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INHERITANCE OF CORN GRASS A MACROMUTATION IN MAIZE, AND ITS POSSIBLE SIGNIFICANCE AS AN ANCESTRAL TYPE*

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INTRODUCTION

From time to time mutations arise which have a more pronounced effect upon the organism than the ones usually studied in genetic investigations. Goldschmidt (1940, 1948) refers to these as macromutations in contrast to the more abundant micromutations. He was the first to emphasize the importance of macromutation in evolution. He questions whether the normal mutations with which we are all familiar, the micromutations, have much if anything to do with the evolutionary process.

Corn grass is definitely such a macromutation. A single gene changes the morphology of the maize plant so completely that it is scarcely recognizable as corn. The leaves are narrow, the plant tillers profusely, the pistillate flowers are located in the axils of the leaves with but a few flowers in a place, and not in a well-defined ear (figures 1, 2 and 3). When first grown the Corn grass plants either had no staminate flowers at all or the flowers were so enclosed in long bracts that no pollen was ever shed. It was maintained by saving open pollinated seed from the heterozygous Corn grass plants. Since the gene is dominant such plants usually segregate in a 1:1 ratio.

Corn grass plants can be propagated vegetatively. A single clump has been divided into sixteen plants and undoubtedly more could have been obtained. This character, along with the seed distribution among many small "ears" on the plant, are two factors that might permit such a plant to become established in the wild state. In a climate free from frost it might be a perennial. Such a plant could then go on for generations with a likelihood that eventually a reverse macromutation might occur and a typical corn plant be produced. To some this may seem too easy a solution to the question of how corn originated. We are not postulating this as the answer, but such a possibility is certainly not unreasonable. In thinking

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FIGURE 1. Corn grass and normal plants in a segregating progeny. Corn grass plants produced a few seeds each, but no pollen. Summer of 1948.

about the ancestor of corn perhaps we have been thinking too much along the line of a corn plant only slightly modified by a series of micromutations, when we should be considering an entirely different type of plant from which corn arose by a single macromutation. If a single gene difference can make plants as dissimilar as corn and Corn grass there is no reason to believe that in some prehistoric time a profusely tillered, perennial, narrow-leaved grass-like plant might not have mutated to a single stalked corn with a well-defined ear similar to types we have today.

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HISTORY OF CORN GRASS

A single plant of Corn grass was first discovered in the summer of 1941 in a field of Lincoln sweet corn hybrid on the farm of A. W. Winter, Passaic County, New Jersey, by Bailey Pepper, entomologist at the New Jersey Agricultural Experiment Station. Dr. Pepper was dusting the experimental plots of sweet corn for control of the European corn borer. He was so struck with the unusual character of the plant that he transplanted it to the greenhouse at New Brunswick and matured a 'small quantity of seed, which was given to Dr. C. M. Haensler, pathologist at the New Jersey Station. Dr. Haensler in turn gave it to me.

It was grown for the first time on the farm of the Connecticut Agricultural Experiment Station in 1945 and was segregating for both Cg and normal



FIGURE 2. Three fairly typical "ears" of Corn grass. Such ears may contain from 0-20 seeds with an average of seven seeds each. These ears were produced on plants similar to those in figure 1. Summer of 1948.

plants in about equal numbers. The normal plants upon selfing produced all normal offspring, while open pollinated seed of the Corn grass gave normal and Cg again in about equal numbers. The Corn grass plants were heterozygous and had received pollen from recessive normal plants.

When first reported Corn grass was referred to as a "second Teopod" (Singleton, 1947) because of some similarity to the original Teopod (Lindstrom, 1925). However, Corn grass is much more striking in appearance than the original Teopod, which would never be mistaken for anything other



FIGURE 3. Three ears produced on a single plant, greenhouse crop of 1948 autumn sowing. Note extreme variation in development of bracts in three ears produced on the same plant.

than corn. The leaves of Teopod are broad and the stalk is about the same height as normal corn. Both inflorescences are considerably modified, however. The seeds are enclosed in long bracts, with the ear much shortened and reduced in size. The tassel of Teopod has few branches and often has a small ear at the base of a single staminate spike. Corn grass, on the other hand, has very narrow leaves unlike normal corn and has much smaller ears with no tassel on many of the plants. Consequently the name of Corn grass seems more appropriate than a "second Teopod." Also the term "second Teopod" should not have been used by us (1947) since Lindstrom (1935) reported two additional cases of mutations to a Teopod type plant. This publication of Lindstrom has but recently come to our attention. One of the illustrations of the new Teopod of Lindstrom appears quite similar to Corn grass. Lindstrom stated "Crosses of the new Teopod-like variants with the original stock of Teopod, indicate that the same basic dominant *Tp* gene is involved." He did not elaborate further on this statement or present evidence of segregating progenies of these crosses, so it cannot be concluded from the data presented that all three of Lindstrom's Teopods were due to the same gene. One was much more similar to Corn grass than to Teopod. Crosses of Corn grass and the original Teopod of Lindstrom

have demonstrated they are not due to the same gene. Evidence is presented later in this paper.

EXPERIMENTAL RESULTS

When Corn grass was first grown at the Connecticut Experiment Station in 1945 it was noted that there were about equal numbers of Corn grass and normal plants in the progeny. Since it was not known whether the mutant was dominant or recessive, seed was saved from both types; selfed seed from the normal plants, and open pollinated seed from the Corn grass plants which produced no pollen. Such open pollinated seed represented a back-cross progeny $Cg/+ \times +$ and produced a progeny segregating for approximately 50 per cent. Corn grass plants. Selfed seed from the normal plants produced only normals, the recessive type.

It was noted in several years that other segregations gave approximately 1:1 ratios. Typical of these are the data for 1950. In these progenies a search was made for a linkage of Corn grass with the following genes, *su*, *pr*, *r*, *wx* and *gl*. No linkage was found with these genes in five different chromosomes. It is significant that none was found with *gl*, which shows only 9.5 per cent. crossing over with Teopod, another bit of evidence indicating Teopod and Corn grass are determined by different genes.

TABLE 1

RATIOS OF *Cg* AND + PLANTS IN BACKCROSSED PROGENIES ($Cg/+ \times +$) 1950

Type of Seed	+ Plants	<i>Cg</i> plants	Total
<i>Pr Su</i>	58	60	118
<i>Y Su wx</i>	39	27	66
<i>pr Su</i>	46	60	106
<i>su</i>	48	45	93
<i>Y Su Wx</i>	52	56	108
	243	248	491
Expected	245.5	245.5	

A chi square test ($\chi^2 = .05$) is unnecessary to tell us there is no serious deviation from a 1:1 ratio.

Table 2 shows F_2 segregations in which *gl* and *Cg* were involved.

TABLE 2

SELFED PROGENIES SEGREGATING $Gl/Cg/gl\ cg$

Row No.	<i>Cg Gl</i>	<i>Cg gl</i>	<i>cg Gl</i>	<i>cg gl</i>	
931-950	174	48	55	15	
955-964	59	21	25	6	
878-889	42	17	22	6	
Total	275	86	102	27	490
Expected	275.6	91.9	91.9	30.6	

Here again there is no significant variation from a 9:3:3:1 ratio indicating that *Cg* is not linked with the *gl*₁ locus, evidence that *Cg* and *Tp* are not allelic. Conclusive proof of this however was obtained from the cross of *Tp/+* × *Cg/+*. The F_2 of this cross gave the following segregation:

TABLE 3
SEGREGATION OF F_2 OF *Tp/+ Cg/+*

Row No.	<i>Tp Cg</i>	<i>Tp +</i>	<i>Cg +</i>	++	
901-905	18	11	12	10	
908-915	<u>19</u>	<u>53</u>	<u>64</u>	<u>29</u>	
Total	37	64	76	39	216
Expected	121.5	40.5	40.5	13.5	

In this instance there is a wide deviation from a 9:3:3:1 ratio. Possibly there is a linkage between *Tp* and *Cg*. If so *Cg* must lie to the right of *Tp* since *Cg* and *gl* show no linkage. Part of the apparent linkage may be due to faulty classification. It was extremely difficult to distinguish *Tp+* and *Tp Cg*. Perhaps we were overcautious in not putting as many in the double dominant class as it deserved. The basis of classification of *Tp Cg* plants was a modification of the *Tp* type of plant. Plants assumed to be *Cg Tp* had a longer ear than ever found on any *Tp* plant without *Cg*. (figures 4, 5 and 6). There was less difficulty in classifying the normal (++) plants although it is possible for modifying genes to affect a *Cg* plant to such an extent that it appears normal. This will be discussed more fully in another section. If the excess of + plants is a real one and not due to modifiers it can scarcely be attributed to linkage since the genes entered the cross in the repulsion phase. The fact that any *Cg Tp* and ++ plants appeared in the F_2 progeny is direct evidence that *Cg* and *Tp* are not allelic. More experimental data are needed to establish a linkage of *Cg*. No indication of linkage was found in tests with genes on the following chromosomes, 4, 5, 7, 9 and 10. The other five chromosomes have not been tested.

ENVIRONMENTAL EFFECT ON MORPHOLOGY OF CORN GRASS

Corn grass grown in the greenhouse in the autumn with no supplemental light is considerably modified in its morphology, assuming an appearance more like normal corn (Singleton, 1949, 1950). Plants from such fall sowings assume a more upright growth, have fewer tillers, produce tassels (in some instances almost like normal corn) and have much broader leaves than field-grown plants. Some of these *Cg/+* plants grown in the greenhouse have been mistaken for normal corn. Upon selfing, these plants have segregated for *Cg* and + thus demonstrating they were genetically *Cg*, also



FIGURE 4. Plant heterozygous for both Teopod and Corn grass.



FIGURE 5. Three tassels on *Tp+Cg+* plant illustrated in figure 4. Note considerable variation in tassel development.



FIGURE 6. Three ears on a $Tp+Cg+$ plant shown in figure 5. Note variation in ears, also one ear considerably longer than those produced on $Tp/+$ plants without influence of Cg gene.

showing how completely the Corn grass plants can be modified, presumably by external growing conditions. It seems that light may have been largely responsible for this marked change, since the plants were grown in the autumn when the days were becoming shorter. The return to a Corn grass-like plant in the January sowings further suggests light, rather than temperature as the controlling factor. The temperature of the greenhouse was thermostatically controlled and remained fairly constant throughout the growing season. However, in Texas, greenhouse-grown plants were upright with few tillers even though supplied with supplemental light (Whaley and Leach, 1950). Their statement seems pertinent, "The relative roles of light and temperature in this tillering response remain to be worked out."

This unusual type of Corn grass plant was first observed in the fall of 1947 when a few plants were grown in the greenhouse of the Connecticut Experiment Station at New Haven. The following year sowings were made at intervals of two weeks beginning September 15 at the Brookhaven Laboratory. This experiment was repeated in 1949 with similar results. Plants from the September sowings were similar to the summer type plants. In October the more erect upright growth commences and persists until about January 1 when the summer type growth reappears. There is a somewhat

gradual transition from the erect type to the typical Corn grass plants. This is illustrated in figures 7, 8 and 9. Figure 10 shows a more striking change that took place in 1949. In this instance the erect plants were more nearly like normal corn.

It is remarkable that such a small change in the environment could produce such a gross morphological effect. Not only is the size and number of tillers affected, but in certain plants a potential floral structure is changed into a completely vegetative part (figures 11 and 12). This is quite characteristic of summer type Corn grass. The change illustrated here occurred on the January 1, 1949, sowing.

GENETIC MODIFIERS OF CORN GRASS

Not only environmental but also genetic modifiers have a pronounced effect on the Corn grass phenotype. When first grown in 1945 and for several



FIGURE 7. Corn grass plants from four different sowings (from left to right) September 15, September 30, October 15 and November 1, 1948. Note more upright growth in later sowings.

years thereafter Corn grass in the summer time never shed any pollen. Any anthers if present were completely enclosed by long bracts and hence produced no pollen. In 1949 and 1950, however, we have had plants that produced fairly normal tassels, though small, and produced plenty of viable pollen. Apparently modifying genes have been introduced into the stock. Mr. Walton Galinat, who has made a comprehensive study of the morphology of Corn grass, has observed these modifiers also. Galinat (1951) crossed Corn grass by stocks differing markedly in maturity and has extracted widely

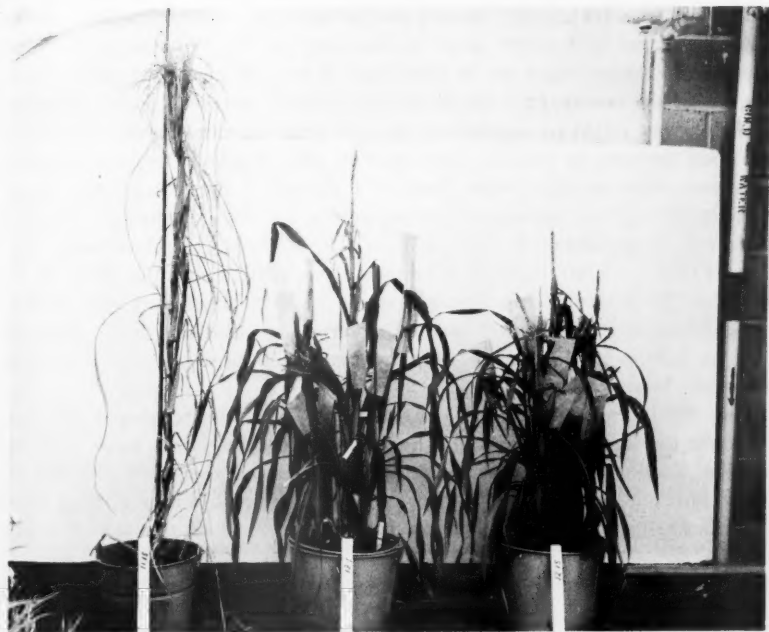


FIGURE 8. Corn grass plants from three sowings, November 15, December 1 and December 15, 1948.

divergent types of Corn grass with striking differences in ear and tassel development when grown under field conditions. Some of his Corn grass plants have produced ears and tassels comparable to normal corn. Apparently the Corn grass character is quite susceptible to modification and is fruitful material for a morphological study.

DISCUSSION

Corn grass is a mutant type whose morphology is grossly altered by a single gene. Meiosis is regular with no indication of chromosomal irregularity. Young plants of Corn grass more closely resemble certain species of common grasses than they do *Zea Mays* L. In fact, Corn grass is so different from corn, especially in the early stages, that students in taxonomy have failed to recognize it as a relative of corn. In Texas, it was so similar to Johnson grass (a serious weed) it was difficult to keep the farm hands from pulling out the Corn grass (Whaley and Leach, 1950).

The question arises whether such mutations as this and the somewhat similar Teopods have any bearing on the question of the type of plant that was the ancestor of maize. In our search for the ancestor have we been looking too diligently for a corn plant only slightly modified, when perhaps

our attention should have been focussed on a grass-like plant such as the Corn grass with its grossly altered morphology? If a difference as large as this can be due to a single gene is there any reason to suppose that even greater differences might not be possible? If to Corn grass we add complete fertility in the homozygous condition along with a perennial habit of growth we have what might be considered an excellent ancestral type. And there are good reasons to believe that species may originate by single macromutations such as this rather than by a series of micromutations. Goldschmidt (1948) has presented the arguments for the importance of macromutations in evolution. We should like to quote Goldschmidt's closing paragraph (1948), "But it will be beneficial to realize that, in view of the failure of Neo-Darwinism to explain evolution occurring above the level of the ecospecies, science will be better served by attacking these problems with an open mind instead of from the standpoint of a school sworn to an inflexible dogma."

Both Weatherwax (1950) and Mangelsdorf (1950) have presented excellent dissertations on the history and the mystery of corn. Both have made extensive studies regarding the ancestry of corn and they do not agree entirely, which is natural when it is considered that no wild type of corn plants have been found. Mangelsdorf is inclined to think of the ancestor as a



FIGURE 9. Corn grass plants from three sowings, December 15, 1948, January 1 and January 15, 1949. Plants from January sowing have all appearances of summer grown plants.



FIGURE 10. Two Corn grass plants on left from October 1, 1949 sowing, those on right from October 15 sowing.

tunicate popcorn being modified from normal corn by the single *Tu* gene. We would like to quote one paragraph (Mangelsdorf, 1950):

So the teosinte theory has become increasingly untenable. Meanwhile the theory that corn originated from pod corn has become more and more plausible. When a modern hybrid form of pod corn is inbred (a process that usually intensifies inherent traits) the result is a plant quite different from ordinary cultivated corn. The ear disappears and the kernels, now borne on the branches of the tassel, are enclosed in glumes, or chaff, as in other cereals. This pure pod corn possesses a means of dispersal, since its seeds are not on a heavy ear but on fragile branches. In the proper environment it could undoubtedly survive in the wild and reproduce itself. It has characteristics like those of many wild grasses; indeed, in its principal botanical features it is quite similar to its wild relative *Tripsacum*. Pure pod corn has virtually all of the characteristics we would expect to find in the ancestral form of corn. Furthermore, it is more than a relative of corn; it is corn—a form of corn that differs from cultivated corn in exactly the way a wild species ought to differ from its cultivated counterpart. Finally, all the hereditary differences between pod corn and cultivated corn are traceable to just one gene on one chromosome. Thus a single mutation can change pod corn to the non-podded form, and it has actually done so in my cultures.

If we were to change the term "pod corn" to Corn grass in this paragraph we believe an even stronger case could be made for the origin of corn. Seed dispersal of Corn grass would be more easily accomplished since the "ears" are much smaller. Also Corn grass can be propagated asexually. The only thing we would have to change in Mangelsdorf's paragraph would be the last



FIGURE 11. Plant of January 1, 1950 sowing showing summer type growth, also showing conversion of floral structure into a vegetative one. Larger view of this in figure 12.

sentence since we have not as yet secured a reverse mutation from Corn grass to normal. Such should be possible as soon as Cg is made homozygous. Preliminary attempts to induce somatic mutations from Cg to normal by use of beta radiation from P^{32} and gamma radiation from Co^{60} did not induce any mutations. Since somatic mutations occur less frequently than germinal



FIGURE 12. Tassel branch of plant of January 1, 1950 sowing showing transformation of a floral structure to a vegetative one. This type of transformation is quite common in plants grown in the summer in the field.

ones it is not surprising that no mutations were found. If the homozygous Cg Cg is not sterile it should be possible to secure a reverse mutation to normal as the mutation must have occurred in the first place, if our theory is correct.

Another cogent reason for assuming that our corn as we know it arose by a single macromutation is that corn is absolutely dependent upon man for support. The primitive Indian would not have made any effort to select among grass-like plants those with a few more seeds than others. However, if a macromutation occurred it would have been recognized immediately and the chances are fairly good that it would have been cared for and propagated. Although it is not possible to obtain proof for this theory of the origin of corn (or any other theory for that matter) it seems possible that a Corn grass-like plant may have been the immediate ancestor.

SUMMARY

Corn grass is a monogenic dominant type of maize that arose by a single macromutation. It has narrow leaves, many tillers and under field conditions hardly ever sheds pollen. It can be propagated asexually and seed is distributed over the plant in many small ears containing but a few seeds each. Several of these characters are those which might be expected in an ancestor of maize. The possibility that a Corn grass type of plant was the ancestor of maize is discussed.

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ANATOMY OF GENE CENTERS¹JACK R. HARLAN²

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The geobotanical work of Nikolai Ivanovich Vavilov is familiar to nearly every biologist. The tremendous mass of material assembled by the institute which he directed for many years made possible a geographic survey of crop plants on a scale which has never been duplicated. In no other country and at no other time has an agronomist had such facilities at his disposal. The work is so monumental that it stands today neither substantiated nor disproved by independent research. Some of his claims have been questioned, but the principal theses of Vavilov are known and appreciated the world over, with the possible exception of his native land where his genius inspired jealousy and his integrity invited liquidation. During the greater part of 1948, the writer was privileged to travel through Turkey on behalf of the Division of Plant Exploration and Introduction of the U. S. Department of Agriculture. This is an area of considerable importance according to the studies of Vavilov (1926, 1935, 1940), and the purpose of this essay is to report certain observations made in the field and to call to the attention of American naturalists some of the opportunities now available for fundamental studies on the problem.

Vavilov's ideas have themselves undergone an evolution of considerable interest. In his classification of 1926 Asia Minor was included in the Asiatic, Mediterranean, Balkan, and Transcaucasian gene centers of wheat including the *vulgare*, *durum*, and *monococcum* groups, and of barley, rye, oats, peas, lentils, chickpeas, bittervetches, vetches, broadbeans and flax. In his classification of 1935 he established eight basic world centers in which most of the varietal wealth of our major crop plants are concentrated. He pointed out that these eight primary centers are separated one from the other by great deserts or mountain ranges. Only in Asia Minor do two basic centers overlap. As a result this part of the world is immensely rich in varietal resources of nearly a hundred species of cultivated plants. By 1940 in one of the last of his essays he had broken up these immense areas into a number of smaller centers of "agro-ecological groups." Of these smaller centers of diversity, Turkey is included at least to some degree in the Syrian group, Anatolian group, Armenian Xerophytic Mountain group, Caucasian Mesophytic High-mountain group, Transcaucasian Humid Sub-

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tropical group and the Mediterranean group. These later groupings are the result of many years of agronomic analyses of the vast amount of material previously accumulated, and of continued explorations of certain primary areas.

It is to be noted that these agro-ecological groups do not coincide exactly with the original concept of center of origin. They represent a further breakdown, a further refinement in the geobotanical pattern of variation. Thus in the overall concept of Vavilov there appears to be a structure in the variation pattern which in itself is subject to study. Observations made by the writer in the field have led him to conclude that the variation pattern may be still further divided into discrete units of relatively small size some of which are intensely active from an evolutionary point of view.

A THRACIAN WHEAT FIELD

An examination of a wheat field in Turkish Thrace provides an education in crop evolution. The typical wheat field here contains a wonderful mixture of forms which according to some classifications would comprise a number of species, many botanical varieties, and dozens if not hundreds of agronomic varieties. Species of *Triticum* found in a single field might include *T. vulgare*, *T. durum*, *T. polonicum*, *T. compactum*, *T. monococcum*, *T. spelta*, *T. turgidum* and others (Gökgöl, 1939). Several so called botanical varieties of each may be present and the agronomic variations are legion. Around the borders of the fields, in the weedrows, the roadsides, the waste spaces and to some extent in the fields themselves, the wild wheat relatives are found in abundance. The borders of many fields are actually carpeted with forms of *Aegilops*, *Haynaldia* and wild forms of *Secale* and *Triticum*. The remarkable array of variants closely resembles certain composite cross populations artificially generated by some of our agronomists in this country, but can hardly be explained on the basis of mechanical mixture alone.

Two population studies in barley may be cited to illustrate the nature of the Thracian wheat populations. In the first study by H. V. Harlan and M. L. Martini (1938) an artificial mixture of varieties was grown for successive generations at widely different localities in the United States. At most stations one or two varieties dominated the mixture within a very few generations, while at one or two stations especially suited to barley culture, all components of the mixture were present after more than ten generations. All varieties were recognizable throughout the experiment and *no new forms were observed*. In the second study (Harlan, Martini and Stevens, 1940) composite cross populations were obtained by crossing 29 varieties in all possible combinations. These populations carried in bulk for eight generations were exceptionally variable and so many new forms and variants were found that they were described as an "understory" in the population. Hundreds of altogether new combinations and some dozens of characters never before observed by agronomists were detected in the advance generations of the composite cross populations (Martini and Harlan, 1942).

GENE MICROCENTERS

With this experimental evidence in mind it seems readily possible to distinguish populations representing mechanical mixtures from those which are to some degree at least actively interbreeding. The Thracian wheat populations appear to be in the latter category. Most wheat populations throughout Turkey, however, belong to the first category being simple mechanical mixtures resulting from a primitive husbandry. Pure fields are rarely found in Asia Minor, but typical grain fields throughout most of Anatolia are represented by a mixture of several forms readily recognizable throughout a local region. The wheat populations of Turkish Thrace are of a different order and represent a remarkable accumulation of variant forms which one might refer to as a gene microcenter.

A similar microcenter was observed in the Kars basin near the Russian border containing entirely different sorts of wheats. The wild relatives consisted mostly of forms of rye and the wheats included *T. persicum* which according to Vavilov is characteristic of the Caucasian Mesophytic High-mountain group of wheats. *T. dicoccum* is more important in the high Kars basin as a secondary crop than *T. monococcum* which is grown on a large scale in Thrace. A third microcenter was observed in southeastern Anatolia in the upper Mesopotamian plain and extending into the Antitoros mountains. All three gene microcenters were characterized by the highly variable type of populations described for Thrace which contrasted sharply with the normal wheat populations throughout the remainder of Turkey.

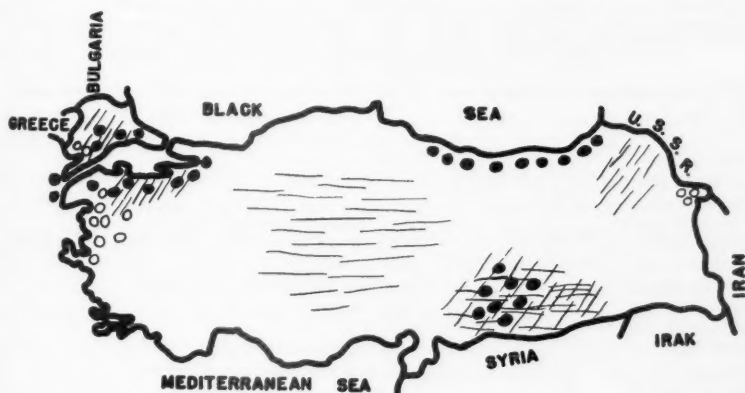


FIGURE 1. Some Turkish gene microcenters:

- ////// Wheats
- Muskmelons
- Pumpkins, cushaws, watermelons, cucumbers, gourds
- New world beans

MICROCENTERS IN OTHER CROPS

Analogous variation patterns were evident in a number of other crop plants, table 1. The locations of microcenters for some of these crops are indicated in figures 1, 2 and 3. It is of special interest to note that several New World domesticates also have microcenter patterns in Asia Minor. This phenomenon is reported in more detail elsewhere (Harlan, 1951).

It is evident, therefore, that the microcenter variation pattern is not uncommon and may in fact be the rule within those broad geographic regions referred to by Vavilov as "centers of origin" or "centers of diversity." Many crops not only exhibit such discrete concentration patterns, but microcenters for many crops frequently coincide. Region 2 as indicated in figure 3 is the richest collecting grounds in Turkey since numerous crops exhibit a concentration of diversity in this area. The second richest collecting area is region 1.

With respect to the Vavilovian concepts of gene centers it is interesting to note that both regions 1 and 2 include an open plain. The Thracian wheat microcenter is so situated and can lay no claim to ecological diversity, remoteness from civilization, isolation, nor even to exceptionally primitive husbandry. Steel plows, grain drills, windmill-type binders are commonly used in Thrace. Agriculture here is as highly developed as anywhere in Turkey, but the unique wheat populations are maintained. Many other sections are agriculturally far more primitive and ecologically much more diverse and yet contain a comparative paucity of genetic variants.

TABLE 1
TURKISH CROPS REPRESENTED BY TRUE GENE MICROCENTER POPULATIONS
OR BY EXCEPTIONAL CONCENTRATION OF VARIANTS IN SMALL
GEOGRAPHIC AREAS

Species	Region (See fig. 3)
<i>Amygdalus</i> spp.	2, 3, 4, 5
<i>Brassica napus</i>	1
<i>Cicer arietinum</i>	1, 2, 4
<i>Citrullus vulgaris</i>	2 and central Anatolia
<i>Cucumis melo</i>	Two centers in region 1; one center in region 5
<i>C. sativus</i>	1, 2
<i>Cucurbita moschata</i>	2 and central Anatolia
<i>C. pepo</i>	2 and central Anatolia
<i>Lens esculentum</i>	1, 2, 3, 4
<i>Lupinus</i> spp.	1 and Mediterranean coast
<i>Malus</i> spp.	2, 3, 4, 5 and Black Sea coast
<i>Medicago sativa</i>	2, 4 and eastern Anatolia
<i>Medicago</i> spp. (annuals)	1 and Mediterranean coast
<i>Onobrychis viciæefolia</i>	2, 3, 4 and eastern Anatolia
<i>Phaseolus vulgaris</i>	1, 2, 3, 4 and Black Sea Coast
<i>Pistacea</i> spp.	2
<i>Prunus</i> spp.	2, 3, 4, 5 and Black Sea Coast
<i>Pyrus</i> spp.	2, 3, 4 and Black Sea Coast
<i>Trifolium</i> spp.	1 and Mediterranean Coast
<i>Vicia faba</i>	1, 2, 3, 4
<i>Vicia</i> spp. (vetches)	1 and Mediterranean Coast
<i>Vitis vinifera</i>	1, 2, 4
<i>Zea mays</i>	Black Sea Coast

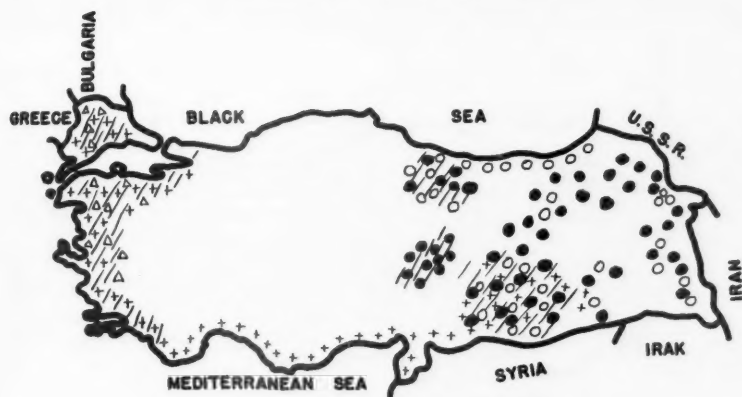


FIGURE 2. Additional centers of varietal diversity:

- ////// Leguminous grains
- O O O Fruit trees
- +++ Annual forage legumes
- • • Perennial forage legumes
- Δ Δ Δ Rape

The nature of these microcenters should be studied by experimental attack. While an intensive survey of field populations in Turkey should yield the best results, a fairly adequate sampling of populations was brought to this country as a result of the expedition of 1948 and some of these materials are presumably available for study (Harlan, 1950).

The emphasis which Vavilov placed upon mountainous regions as the home of agriculture, upon mountain barriers as factors in isolation, upon remoteness from civilization and primitiveness of agriculture in the gene centers he described has probably led most agronomists to conclude that a gene center is a museum of archaic and primitive types which have been preserved in the isolation of hidden mountain valleys by the primitive husbandry of primitive people. This concept may have some application in some regions, but does not represent the whole story. Areas of varietal diversity may be found on the plains or in the mountains, remote from civilization or near to it. They may be very ancient or quite recent in development as indicated by the secondary centers of new world crops in Turkey. The fundamental characteristic which is common to all centers is that evolution is proceeding at a rapid pace *now*. As with all crop plants this evolutionary development is continually assisted or directed by the activity of man. No form of husbandry is too primitive or too specialized to have its effect upon the development of crop plants. Selections are made, natural hybrids occur; the very act of reaping and sowing favors one component of a population over another. New forms arise and are preserved or discarded or left to their fate under competition of a mixed population. It appears, therefore, that agronomists would be justified in accepting a more dynamic concept of a gene center

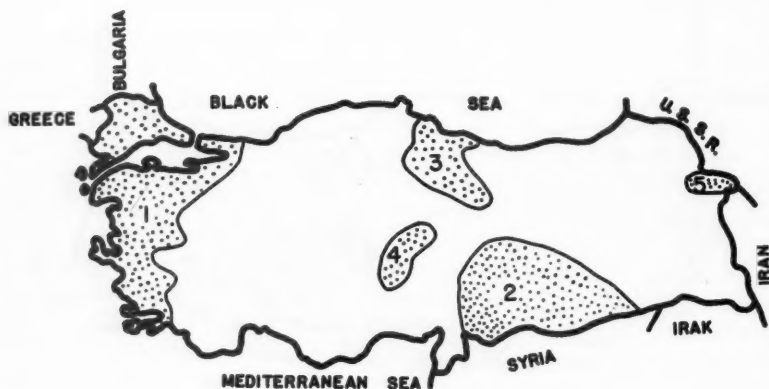


FIGURE 3. Areas of greatest crop diversity and varietal wealth:

- Region 1. Wheats, muskmelons, rape, beans, lentils, chickpeas, broadbeans and annual forage legumes (vetches, lupines, clovers).
- Region 2. Wheats, tree fruits, grapes, pumpkins, cushaws, watermelons, cucumbers, gourds, beans, lentils, chickpeas, broadbeans and both annual and perennial forage legumes.
- Region 3. Fruit trees, beans, lentils, broadbeans and forage legumes.
- Region 4. Fruit trees, grapes, beans, lentils, chickpeas, broadbeans and forage legumes.
- Region 5. Fruit trees, muskmelons and forage legumes.

than Vavilov indicated, and that a center of origin as conceived by Vavilov may actually consist of an aggregate of small microcenters in which the present forms are continuously in the process of origination.

SUMMARY

Observations made by the author during an expedition to Asia Minor in 1948 led him to conclude that the geographic regions referred to by N. I. Vavilov as "centers of origin" or "gene centers" have a structure which is subject to study. Such geographic regions include areas of actual varietal paucity as well as small regions of enormous varietal wealth. The small areas in which is concentrated the varietal diversity of a crop are referred to as gene microcenters. Microcenters of a number of crops frequently coincide. Microcenters may be located on the plains or in mountainous regions, near civilization or remote from it; husbandry may be very primitive or moderately advanced. The one characteristic which is common to all microcenters is that evolution is proceeding at a rapid rate at the present time. An intensive survey of microcenter populations should yield immensely valuable information not only to the student of evolution but also to the agronomist and plant breeder.

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DUPLICATE GENES IN MAIZE

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Duplicate and triplicate factor ratios for various characters have long been known in tetraploid and hexaploid oats and wheat. Duplicate factors have also been reported in a number of known amphidiploids such as *Nicotiana tabacum* (Clausen and Cameron, 1950). The occurrence of 15:1 and 63:1 ratios in polyploid plants is presumptive evidence that some genes are in duplicate or triplicate and that identical or similar loci are present in the different genomes. The recent synthesis by McFadden and Sears (1946) of a vulgare wheat furnishes conclusive evidence that this hexaploid does consist of three different genomic sets of seven chromosomes each. Stadler's (1929) finding of a low induced mutation rate in tetraploid and hexaploid oats and wheat as compared to the much higher mutation rate in the diploid species likewise indicates a considerable duplication of loci in these plants. If 15:1 and 63:1 ratios are evidence of duplicated or triplicated loci in polyploids, do similar ratios in essentially diploid plants such as maize also indicate duplicated loci? That duplications played an important role in the evolution of the maize plant, and indeed in all organisms (Metz, 1947), can hardly be questioned, but it is possible that once homologous loci could become so differentiated by mutation that any trace of their former homology would disappear. Although Sprague (1932) has shown that 15:1 ratios cannot, at least for the inheritance of scutellum color in maize, be taken as critical evidence of duplicate genes, it appears likely that duplicate and triplicate factor inheritance is very suggestive of duplicated loci.

At least 14 cases of duplicate factor, two of triplicate and one of quadruplicate factor inheritance have been reported in maize (see Emerson, Beadle and Fraser, 1935). Unfortunately linkage determinations of duplicate loci are so arduous that nothing is known of the location of many of the sets. In eight of the 14 cases of duplicate factor inheritance the linkage relations of both loci are unknown while in three (xn_1, xn_2 , zg_1, zg_2 , so_1, so_2) one of the members of each set has been assigned to a specific chromosome, the other being unplaced. One of the genes of the xn_1, xn_2 set is in chromosome 10; in both the zg_1, zg_2 and so_1, so_2 sets one gene of each set has been located in chromosome 9 although the published data do not permit a precise determination of the map position. A rather surprising situation is found in the remaining three sets in that the fr_1, fr_2 loci are both in chromosome 7 (Jenkins and Pope in Emerson et al., 1935); the w_1, w_2 genes are both in chromosome 6 (Demerec, 1923); and the au_1, au_2 factors both lie in chromosome 9 (Eyster, 1929 and Emerson et al., 1935). Modified 15:1 ratios result when the duplicated loci are situated in the same chromosome, the observed ratio being a

function of the recombination percentage between the two genes. According to Demerec the w_3 and w_4 genes lie approximately 22 recombination units on either side of the Y_1 locus. Eyster's data place au_1 about 11 units to the right of Wx in chromosome 9, while Jenkins and Pope's data locate r_1 ten units to the right of ij and r_2 15 units to the left of ra_1 and gl_1 in chromosome 7. If duplicate factor inheritance be accepted as indicative of the presence of duplications two conclusions can be drawn from the above data: (1) a disproportionately large number of the located duplicate factors lie in chromosome 9, and (2) inasmuch as a minimum of three of the 14 cases of duplicate factor inheritance have both loci situated in the same chromosome it appears that intra-chromosomal duplication was relatively more frequent than inter-chromosomal.

Maize may be an ancient amphidiploid and, if so, could have homologous segments in duplicate. Two relatives of maize, Coix and Sorghum, have species with a monoploid set of five chromosomes, and it is possible, as Edgar Anderson has recently emphasized, that maize originated from a cross of two five-chromosome species. The observation of occasional bivalent formation in monoploid plants is perhaps due to pairing between homologous segments in different chromosomes although chiasmata formation following non-homologous association might be responsible. Perhaps the most pertinent evidence for duplications is the disproportionate distribution of mutant genes in certain chromosomes, particularly chromosome 9. The long arm of 9 is twice the length of the short arm, yet a great majority of the thirty-odd mutants lie in the short arm. This non-random distribution has been interpreted as indicating the presence of redundant chromatin in the long arm (Anderson, 1938, Rhoades, 1945). It is possibly significant in this connection that four of the nine located duplicate loci are in chromosome 9 although in only one instance (au_1, au_2 set) are the data sufficiently exact to place the au_1 gene in the long arm of 9.

A recent analysis of a new set of duplicate genes has disclosed evidence which is of some import to the above discussion. A viable pale green phenotype is produced when both pg_{11} and pg_{12} are homozygous recessive. The expected 15:1 ratios are found upon selfing $Pg_{11}pg_{11}Pg_{12}pg_{12}$ plants as are 3:1 ratios in F_2 populations if one locus is homozygous recessive and the second is heterozygous. The data in tables 1 and 2 place pg_{11} in chromosome 6 between the Y_1 and Pl loci. The summary of all linkage data gives an average recombination value of 21 per cent between Y_1 and pg_{11} . The pg_{12} locus in chromosome 9 has a mean recombination percentage of 10 with the Wx locus. The data in table 3 show that the linear order is $Sb-Wx-Pg_{11}$. According to Anderson and Randolph (1945) the centromere of chromosome 9 is not much more than two recombination units to the right of Wx . It follows, therefore, that the pg_{12} gene lies in the long arm of chromosome 9. Eyster's data placed au_1 about 11 units to the right of Wx . Even though recombination values are subject to considerable variation it seems highly probable that the pg_{12} and au_1 genes, both members of duplicate sets, lie close to one another in the long arm of chromosome 9—i.e., the arm which was suspected

TABLE 1

BACKCROSS DATA FROM CROSS OF $Y_1-Pg_{11}-Pl/y_1-pg_{11}-pl$ Pg_{12} pg_{12} INDIVIDUALS BY $y_1-pg_{11}-pl$ pg_{12} POLLEN. THE Y_1 AND Pl LOCI GIVE 1:1 RATIOS BUT THE PALE GREEN CHARACTER APPEARS IN A 3:1 RATIO SINCE DUPLICATE GENES ARE INVOLVED IN ITS EXPRESSION.

Y	y	Y	y	Y	y	Y	y	
Pg	pg	pg	Pg	Pg	pg	pg	Pg	
Pl	pl	pl	Pl	pl	Pl	Pl	pl	Σ
661	319	92	176	178	40	3	337	1806

$Y_1-Pg_{11} = 20.9\%$ recombination
 $Pg_{11}-Pl = 9.5\%$ recombination
 linear order is $Y_1-Pg_{11}-Pl$

of possessing redundant chromatin. Demerec's data place either w_3 or w_8 22 recombination units to the right of Y_1 in chromosome 6, which is nearly the same map position found for the pg_{11} gene. It can hardly be fortuitous that the au_1 and pg_{12} genes lie in the same region of chromosome 9 and that the w_3 or w_8 and pg_{11} genes are close together in chromosome 6. It appears probable that the long arm of 9 has a duplicated segment which includes the au_1 and pg_{12} loci, and that there is a duplication in the long arm of chromosome 6 between Y and Pl which carries either w_3 or w_8 and pg_{11} . It might be predicted that nests of duplicate loci will be found in both of these chromosomes when now-unplaced duplicate sets are involved in appropriate linkage tests, although it by no means follows that members of duplicate sets will be restricted to these two chromosomes.

Although the available evidence shows that the au_1 and pg_{12} genes are located in the long arm of chromosome 9 it does not follow that this arm is composed solely of redundant chromatin. Spores deficient for segments of the long arm of 9 are produced by adjacent segregations in plants heterozygous for reciprocal translocations. These deficient spores abort, hence

TABLE 2

BACKCROSS DATA FROM CROSS OF $Y_1-Pg_{11}-Pl/y_1-pg_{11}-pl$ pg_{12} pg_{12} INDIVIDUALS BY $y_1-pg_{11}-pl$ pg_{12} POLLEN. THE pg_{12} LOCUS IS HOMOZYGOUS RECESSIVE SO A 1:1 RATIO IS OBTAINED. THE POORER VIABILITY OF THE PALE GREEN PLANTS ACCOUNTS FOR THE DEFICIENCY OF THE PALE GREEN PHENOTYPE.

(0)	(0)	(1)	(1)	(2)	(2)	(1-2)	(1-2)	
Y	y	Y	y	Y	y	Y	y	
Pg	pg	pg	Pg	Pg	pg	pg	Pg	
Pl	pl	pl	Pl	pl	Pl	Pl	pl	Σ
128	93	32	23	27	11	3	1	318

$Y_1-Pg_{11} = 18.6\%$ recombination
 $Pg_{11}-Pl = 13.2\%$ recombination
 linear order is $Y_1-Pg_{11}-Pl$

TABLE 3

F₂ DATA FROM SELF-POLLINATION OF $\frac{Y_1 Pg_{11}}{y_1 pg_{11}} \frac{Sb Wx Pg_{12}}{sb wx pg_{12}}$ PLANTS.

The Y_1 locus in chromosome 6 is independent of the Sb and Wx loci in chromosome 9 but Y_1 , Sb and Wx all show linkage with the pale green character since it is due to duplicate genes, one of them being in chromosome 6 and the second in chromosome 9.

Y	Y	Y	Y	Y	Y	Y	Y	y	y	y	y	y	y	y	y	
Sb	Sb	sb	sb	Sb	Sb	sb	sb	Sb	Sb	sb	sb	Sb	Sb	sb	sb	
Wx	Wx	Wx	Wx	wx	wx	wx	wx	Wx	Wx	Wx	Wx	wx	wx	wx	wx	
<u>Pg</u>	<u>pg</u>	<u>Pg</u>	<u>pg</u>	<u>Pg</u>	<u>pg</u>	<u>Pg</u>	<u>pg</u>	<u>Pg</u>	<u>pg</u>	<u>Pg</u>	<u>pg</u>	<u>Pg</u>	<u>pg</u>	<u>Pg</u>	<u>pg</u>	$\Sigma = 1552$
810	6	119	0	87	13	123	17	252	7	30	2	17	18	23	28	

$Y_1-Pg_{11} = 22\%$ recombination
 $Sb-Pg_{12} = 25\%$ recombination
 $Sb-Wx = 22\%$ recombination
 $Wx-Pg_{12} = 7\%$ recombination
 $Y_1-Sb = 50\%$ recombination
 $Y_1-Wx = 48\%$ recombination
 linear order is $Sb-Wx-Pg_{12}$

this arm carries loci essential for normal gametophyte development which are not present in duplicate in other chromosomes of the haploid complement. Perhaps both the failure to find more mutant genes in the long arm and the abortion of deficient spores can be explained by intra-chromosomal duplications but this is highly speculative.

The data of table 3 are unusual in one respect. The Y locus in chromosome 6 shows 22 percent recombination with the pale green character and the Wx locus in chromosome 9 has seven percent recombination with the same character yet the Y and Wx loci are independent of one another. This situation is, of course, wholly explicable since the pale green phenotype depends upon duplicate factors. The linkages of Y and Wx with the same pale green character are due to the fact that each is linked with one of the two members of a set of duplicate genes.

Although it may be difficult to differentiate between them in practice a fundamental distinction exists between a suppressor and a duplicate gene. As its name signifies a duplicate gene is similar or identical to another locus; the recessive phenotype is not expressed unless the recessive alleles of both loci are homozygous. A suppressor gene inhibits or nullifies the effect of another, non-homologous locus. Houlahan and Mitchell (1947) were able to discriminate between a suppressor and a duplicate gene in *Neurospora*. In their study of mutations which control the synthesis of pyrimidine they found three different alleles at one locus. A mutation occurred which suppressed the effect of the three alleles—i.e., strains with both the suppressor mutant and a pyrimidineless gene had a normal growth habit on medium lacking pyrimidine. They believe that the suppressor mutant is not a duplicate of the wild type allele of the pyrimidine locus

since strains lacking the suppressor but carrying the wild type allele grew normally in the presence of arginine while strains with the suppressor mutant and the pyrimidineless allele were inhibited.

Lindstrom (1921) found that neither the L_1 nor the l_1 alleles had any effect on chlorophyll synthesis in maize. Their action was manifest only in the presence of other mutant genes. For example, seedlings homozygous for the white seedling gene w_1 were cream-white if the dominant L_1 allele was present but were yellow if the recessive l_1 allele was homozygous. The action of l_1 was not restricted to a specific w gene but modified the effect of a number of white and virescent genes. While nothing is known of the way in which the l_1 gene acts in producing a yellow rather than a white phenotype it may be considered as a partial suppressor of a large number of mutant loci. This behavior is in striking contrast to the specific action of the suppressor genes of *Drosophila*.

An unusual situation has been reported by Everett (1949) in maize. The recessive gene cl_1 produces seeds with light colored endosperm which when germinated give rise to albino seedlings. Everett considers cl_1 to be a pleiotropic gene. The Cl_2 allele, which segregates independently of cl_1 , modifies the chlorophyll effect of cl_1 but has no effect on endosperm color. Seedlings of $cl_1 cl_1 cl_2 cl_2$ constitution are albinos, those of $cl_1 cl_1 Cl_2 cl_2$ are light green while those of $cl_1 cl_1 Cl_2 Cl_2$ constitution are pseudo-normal. The pseudo-normal seedlings are green but die in the seedling stage as do the light green seedlings. The Cl_2 allele acts as a partial suppressor of cl_1 . A third gene Cl_3 likewise has no effect on endosperm color of cl_1 kernels but $cl_1 cl_1 Cl_3 cl_3$ and $cl_1 cl_1 Cl_3 Cl_3$ plants are both green and viable. It was concluded that the Cl_3 gene is a semi-duplicate of the Cl_1 locus.

Aside from duplicate factor inheritance, further evidence of duplications in maize is Laughnan's (1949) recent demonstration that the A_1^b allele is composed of two separable components. His results are in some ways comparable to those found for the lozenge and Star-asteroid loci in *Drosophila* (Green and Green, 1949, Lewis, 1945) where, at least for Star-asteroid, there is cytological evidence of a duplicated locus. McClintock has interpreted (quoted in Metz, 1947) her data on pseudo-alleles near the end of the short arm of chromosome 9 of maize as indicating the presence of a series of similar or identical genes which have arisen through duplication of individual loci or short segments.

That the architecture of the germ plasm of maize contains duplicated regions can hardly be doubted but whether or not they represent vestiges reflecting an ancient amphidiploid origin or represent later occurring duplications cannot be decided at this time.

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METABOLIC TYPES AND GROWTH TYPES¹

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The problem of the relation between metabolism and body size is one of the classical topics of physiology. It is usually treated in terms of the surface rule, established by Rubner in 1883, and heralded by earlier work of Sarrus and Rameaux, Bergmann and Richet. The surface rule states that the metabolic rate per unit weight decreases with increasing size, but is constant per unit surface. Rubner explained the surface rule in terms of homeothermy: Since all warm-blooded animals heat their bodies to a temperature of approximately 37°C, and since heat output takes place on the body surface, the same number of calories must be produced per unit surface. Even in recent discussions (Brody, 1945; Kleiber, 1947; Krebs, 1950) homeothermic animals are taken almost solely into consideration. It is necessary, however, to consider the problem on the broader basis of comparative physiology.

Such investigation has been carried through by the author and his co-workers (Bertalanffy, 1942 et seq.). The main results of this re-examination of the problem are:

(1) Recent investigation shows that the surface rule also holds for poikilothermic vertebrates and certain invertebrates. The rule as such is of a wide application, but current explanations, especially the explanation based on homeothermy, are too restricted.

(2) On the other hand, there are many classes of animals for which the surface rule does not apply.

(3) Thus we came to the statement of several *metabolic types* in respect to the relation between metabolic rate and body size. So far, three metabolic types have been distinguished.

In the first type, metabolic rate is proportional to a surface or to the $\frac{2}{3}$ power of weight. Representatives of this type are fish, but also certain invertebrates, such as isopod crustaceans, according to our own investigations (Bertalanffy and Müller, 1943c), mussels (Weinland, 1919; Ludwig and Krywienczyk, 1950), and *Ascaris* (Krüger, 1940). As an example, metabolic rates in the sow bug, *Armadillidium*, are presented in table 1. The rate of oxygen consumption decreases per unit weight, but is constant per unit surface, expressed as the $\frac{2}{3}$ power of weight. The most important question is, of course, whether the surface rule applies to mammals. This

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TABLE 1
CO₂ PRODUCTION OF ARMADILLIDIUM PALLASII
(21°, mean values of 55 determinations)

weight in mg	15	33	50	100	160
cmm O ₂ /h	3,0	5,2	7,2	11,2	15,2
per gm/h	200	174	144	112	94
per unit surface (W ^{2/3})/h	48,5	54,2	53,0	49,8	51,6

question has been much discussed in recent years (Brody, 1945; Kleiber, 1947). In our work, the surface rule appears to be valid in the rat, comparing metabolic rates in individuals of different size (unpublished).

The second type is entirely different. Here metabolic rate is directly proportional to weight. The most important cases are insect larvae (Bertalanffy and Müller, 1943a; Teissier, 1931), and insects in interspecific comparison (Kittel, 1941), but equally hemimetabolic insects (Bertalanffy and Müller, 1943b) belong to this type. The example given is the walking stick, *Dixippus morosus*. Table 2 shows the constancy of metabolic rates per unit weight. Also land snails of the order Helicidae (intraspecific comparison: Bertalanffy and Müller, 1943a; interspecific comparison: Liebsch, 1929), and annelids, such as the earth worm (Bertalanffy and Müller, 1943c) belong to this type.

TABLE 2
OXYGEN CONSUMPTION OF DIXIPPUS MOROSUS
(20°, mean values of 20 determinations)

weight in mg	8	130	250	450	630	850
cmm O ₂ /h	2,0	30,6	60,7	113,2	154,8	206,6
per gm/h	250	236	243	252	245	242

In the third type, metabolic rate is intermediate between weight proportionality and proportionality to surface. To this type belong pond snails such as *Planorbis* and *Limnaea*, and Planarians (Bertalanffy and Müller, 1943a). Table 3 demonstrates that metabolic rates decrease with respect to weight, but increase with respect to surface.

Now just as there are different types of metabolism, there are also different types of growth. The most common type of animal growth is that growth rates continually decrease with time and finally the organism reaches

TABLE 3
OXYGEN CONSUMPTION OF PLANORBIS SPEC.
(23°, mean values of 48 determinations)

weight in mg	30-35	58-62	90-100	140	190-200
cmm O ₂ /h	2,3	3,9	5,4	7,3	9,5
per gm/h	69	65	56	52	48
per unit surface (W ^{2/3})/h	22,9	25,1	26,1	27,0	28,2

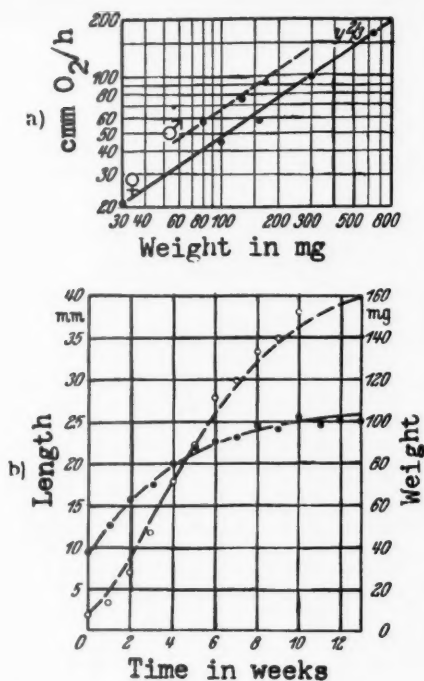


FIGURE 1. (a) Respiration at 20°C (mean values of 85 determinations); (b) growth curves in the fish, *Lebistes reticulatus*. Calculation of linear growth: ———; of weight growth: - - - - - . Equation for linear growth:

$$x = X - (X - x_0)e^{-kt},$$

for weight growth:

$$y = \left[\sqrt[3]{Y} - (\sqrt[3]{Y} - \sqrt[3]{y_0}) \right] e^{-kt}.$$

x, y = length, weight at time t ; X, x_0, Y, y_0 = final and initial length and weight, respectively; $k = K/3$.

a steady state in the adult. There are, however, also other types of animal growth. It appears that it is possible to establish a *strict connection between growth types and metabolic types* with respect to the dependence of metabolic rate on body size. We give typical examples for these types.

In the first type, metabolism is surface-proportional. Figure 1 shows metabolism and growth in the guppy, *Lebistes reticulatus*. Metabolic rates are presented in allometric or log-log plot against weight. In the case of surface proportionality, the allometric line has a slope of $\frac{1}{3}$. The corresponding growth curve of this type is characterized as follows: (1) Growth rates are decreasing and the growth process attains a steady state; (2) the curves of growth by weight and linear growth show characteristic differences: the curve of weight growth has a point of inflection at about $\frac{1}{3}$ of the final weight, the curve of linear growth shows simple exponential decrease.

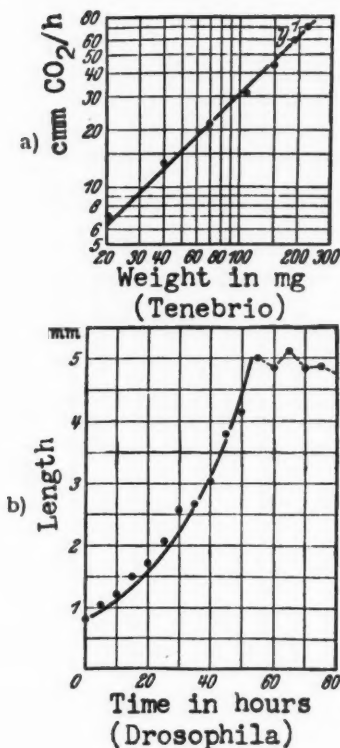


FIGURE 2. (a) Respiration at 20°C (mean values of 84 determinations); (b) growth (data of Alpatov) in insect larvae. Growth equations:

$$x = x_0 e^{ct}; y = y_0 e^{3ct}.$$

x, y = length, weight at time t ; x_0, y_0 = initial length, weight; c = constant.

As an example of the second type, we give metabolism and growth in insect larvae (figure 2). In this type, metabolic rate is weight-proportional and therefore gives an allometric line of slope 1. Growth is here exponential, that is, growth rates always increase and no steady state is reached. Growth is only intercepted by a sort of crisis which is represented by metamorphosis in insect larvae, by seasonal cycles in land snails, which, as said before, also belong to this type.

In the third type, metabolic rate stands between proportionality to weight and proportionality to surface, giving an allometric line of a slope between $\frac{2}{3}$ and 1. Our example is the pond snail, *Planorbis* (figure 3). In this case the curve of weight growth does not differ much from that of the first type, but the curve of linear growth is characteristic. While in the first type the curve has no point of inflection, it has one in the third type.

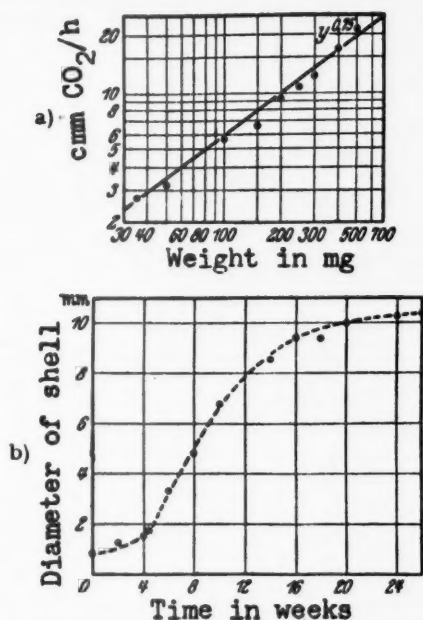


FIGURE 3. (a) Respiration at 23°C (mean values of 67 determinations); (b) growth in *Planorbis spec.* The theoretical point of inflection is indicated by an asterisk (*).

The relation between metabolic types and growth types can be explained, and many surprising predictions can be made on the basis of a theory on animal growth advocated by the author (1941, 1942, 1948, 1949). Growth is considered to be the result of a counteraction of anabolism and catabolism of building materials, according to the following basic expression:

$$\frac{dy}{dt} = \eta y^m - \kappa y^n.$$

In words: the change of body weight y is given by the difference between the processes of building up and breaking down: η and κ are constants of anabolism and catabolism respectively, while the exponents m and n indicate that the latter are proportional to some powers of body weight y .

Catabolism is, at least in a first approximation, proportional to weight so that the exponent n can be set equal to 1. Inserting for the exponent of anabolism m that value which is found for size dependence of metabolism, the types of growth automatically follow and the formulas for laws governing the several growth types can be deduced. The mathematical formulations are given elsewhere (Bertalanffy, 1941, 1942). It can be said, however, that these deductions have been found valid in all cases sufficiently investigated. Ludwig (1950) who is one of the most active workers in this field in Continental Europe, states that "no contradictions were found in his experiments to Bertalanffy's theory."

The relations found are summarized in table 4, which indicates the metabolic types, the corresponding growth types, and examples investigated. It is hoped that these investigations will lead to a comparative physiology of metabolism and growth.

TABLE 4
METABOLIC TYPES AND GROWTH TYPES

Metabolic type	Growth type	Examples
I. Respiration <i>surface-pro- portional</i>	(a) Linear growth curve: attaining <i>without inflexion</i> a steady state. (b) Weight growth curve: <i>sigmoid</i> , attain- ing, with inflexion at c. $\frac{1}{3}$ of final weight, a steady state	Lamellibranchs, fish, mammals
II. Respiration <i>weight-pro- portional</i>	Linear and weight growth curves <i>exponential</i> , no steady state attained, but growth intercepted by metamorphosis or seasonal cycles	Insect larvae, Orthoptera, Helicidae
III. Respiration <i>in- termediate be- tween surface- and weight- proportionality</i>	(a) Linear growth curve: attaining <i>with inflexion</i> a steady state. (b) Weight growth curve: <i>sigmoid</i> , similar to I(b)	Planorbidae

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A SURVEY OF CHEMICALS FOR MUTAGENIC ACTION ON *E. COLI*¹

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INTRODUCTION

At present we have available two good methods, using *Escherichia coli*, for the quantitative study of spontaneous mutability and of the mutagenic action of various agents such as radiations and chemicals. The older of the two methods deals with mutational changes from phage sensitivity to phage resistance, and the more recently developed one (Demerec, 1951; Bertani, 1951) with back-mutations from streptomycin dependence to nondependence. In both cases the mutants can be easily classified; very large numbers of bacteria may be tested; and slight increases in mutation rate can be easily detected.

After the streptomycin method had been developed we undertook extensive tests of a number of chemicals for mutagenicity, using mainly this method because it is the simpler of the two.

SELECTION OF CHEMICALS

Previous work by Witkin with *E. coli* (Witkin, 1947; Demerec, Wallace, and Witkin, 1948) had indicated that compounds exhibiting quite different properties may all be mutagenic. Using the phage method, she found that sodium desoxycholate, pyronin, acriflavine, caffeine, and colchicine acted as mutagens, but detected no mutagenic effect in tests with methyl green, basic fuchsin, and methylcholanthrene endosuccinate.

In planning the present survey, therefore, we prepared a list of chemicals, including representatives of widely separated groups, from simple inorganic and organic compounds to complex organic chemicals. Before the scheduled tests had been completed, however, we found that certain ferrous and manganoous salts are very potent mutagens, and our attention was thus diverted to a detailed study of these compounds. Since it will require some time to

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carry this out, the data so far available from the survey are being summarized here.

MATERIALS AND METHODS

The "streptomycin" method for detection of induced mutations has been fully described by Bertani (1951). Only technical details will be given here.

Strains B/Sd-4/1,3,4,5 and B/Sd-4/3,4 of *E. Coli* were used throughout this work. For every experiment, bacteria were grown for 24 hours at 37°C in an aerated broth culture containing 10 micrograms of streptomycin per milliliter. They reached a saturation titer of approximately 2 to 3×10^8 cells per milliliter. Each culture was started from an inoculum, usually large, taken from streptomycin-agar slants kept in a refrigerator. Before treatment the bacteria were washed in saline and resuspended in distilled water. A sample of the new suspension was added to the desired solution of chemical in distilled water, and incubated at 37°C for a certain period of time; no growth occurs under these conditions. Another sample of the same suspension was added to an equal amount of distilled water and incubated for the same period of time, as a control. At the end of the treatment period, both treated and control suspensions were assayed by plating suitable dilutions on streptomycin-agar plates. At the same time they were plated (0.1 ml per Petri dish), either undiluted or diluted not more than 1:10 in plain broth, onto a number of streptomycin-free plates, using a glass spreader and a turntable. The assay plates were incubated for 48 hours, after which it was possible to count the colonies and calculate the titers of the two suspensions at the end of treatment, and the percentage of survivors. The streptomycin-free plates (mutant plates) were incubated for at least six days. After this time the colonies were scored, and the frequency of mutants calculated by dividing the number of colonies by the number of (viable) bacteria plated.

The length of the period of incubation in distilled water does not appreciably affect the proportion of mutations in the controls. In all but a few exceptional cases, nevertheless, the control suspensions were incubated for the same period of time as the chemically treated suspensions.

The degree of crowding of the dependent bacteria on the mutant plates would be expected to affect the observed frequency of mutants, as it influences the extent of residual growth and thus the chance that a mutant cell will develop into a visible colony. This effect is slight, however, when the bacteria plated have been raised in 10 micrograms of streptomycin per milliliter.

The chemical treatment also may limit the extent of residual growth, causing a spurious lowering of the frequency of mutants in the treated series. This may explain why, in experiments with some chemicals, the treated series showed frequencies lower than those of the controls.

Several of the chemicals used cause the formation of clumps of bacteria during treatment. If these clumps have not dissolved by the time the treated suspension is assayed to determine survival, the observed survival value

will be too low, and consequently the calculated mutation frequency too high. Therefore, except in the very first experiments done (*i.e.*, those with sulfuric, nitric, and hydrochloric acids, which in any case were not found to be mutagenic), the treated suspensions were always examined under the microscope before plating, and discarded if clumping was detected. By varying the concentration of chemical and the length of treatment, it was possible to eliminate clumping in many cases.

In a few scattered experiments (and in most of those done with phenol and formaldehyde) the dependent bacteria were grown in 25 instead of 10 micrograms of streptomycin per milliliter. It is known that this results in greater residual growth and therefore a larger number of spontaneous mutants per plate. This explains why in some experiments the spontaneous mutant frequencies were higher than average.

RESULTS

The results of a number of experiments for each chemical are reported in table 1. The concentration of the chemical shown in column 2 is the effective concentration at the time of treatment (*i.e.*, corrected for the addition of the bacterial suspension in distilled water). Number of plates (column 5) is the number of streptomycin-free plates used for the scoring of mutants. Total number of bacteria (column 6) is the number of viable dependent bacteria put on each plate, multiplied by the number of plates used. Number of mutants per 10^8 bacteria (column 7) is the mutant frequency, calculated by dividing the total number of colonies scored in an experiment by the total number of bacteria. The total number of mutant colonies is not given in the table, but can easily be calculated by the inverse operation. From it, one can determine the average number of mutants per plate, which in the experiments reported here was rarely larger than 10-20. Assuming a Poisson distribution, the variance can be estimated and the statistical significance of the results evaluated. Sometimes the same controls were common to several treated series; and in these cases the number of plates and total number of bacteria are reported only once for the controls. Different experiments done with each chemical are listed in order of decreasing survival.

Inorganic acids. Tests made with sulfuric, orthophosphoric, nitric, and hydrochloric acids gave negative results. Under certain conditions, all four of these compounds produced clumping of the treated bacteria, and this explains why in some experiments the values obtained for mutant frequency were higher in the treated series than in the controls. Negative results for hydrochloric acid had also been obtained in earlier experiments with *Drosophila* (Goldat and Beliaieva, 1935).

Boric acid was the only inorganic acid tested that gave positive results. In all experiments but one, the treated series showed an increase in mutation frequency. No clumping was ever observed among bacteria treated with boric acid.

Alkalies. Sodium hydroxide and potassium hydroxide were tested, with inconclusive results. Under the conditions of these experiments they never produced clumping.

Ammonia. Ammonia had been used in experiments with *Drosophila* by Lobashov and Smirnov (1934) and Lobashov (1937), and found to have mutagenic action. In the tests reported here, ammonia showed definite mutagenic activity in experiments where the proportion of survivors was lower than 2 per cent. Apparently similar positive results were obtained with ammonium chloride; they are not reported here, however, because clumping could not be avoided after treatment with this chemical.

Hydrogen peroxide. Attention was first drawn to the mutagenic activity of peroxides by the work of Stone, Wyss, and Haas (1947) on the indirect effect of mutation-inducing radiations on bacteria. Dickey, Cleland, and Lotz (1949), using *Neurospora*, studied the mutagenic potency of hydrogen peroxide and of a number of organic peroxides, and obtained positive results. In our experiments also, hydrogen peroxide was clearly shown to be mutagenic.

Metal salts. Mercuric chloride, silver nitrate, copper sulfate, and sodium silicate were tested. Only copper sulfate showed indications of mutagenic effect in the lower range of survival. Mutagenic potency had already been demonstrated for this salt by Magrzhikovskaja (1936) and Law (1938) in experiments with *Drosophila*. The results reported for mercuric chloride and silver nitrate do not exclude the possibility of their having mutagenic activity at the lowest survival levels, because it was extremely difficult to obtain survivals below 1.0 per cent and yet not so low as to fall beyond the range of sensitivity of the method.

Organic acids. Lobashov and Smirnov (1934) and Lobashov (1937) had treated *Drosophila* with acetic acid, but failed to detect any mutagenic activity of this substance. In our experiments, acetic, formic, and lactic acids were tested. The first two showed mutagenic activity. Some of the experiments with lactic acid indicated a weak mutagenic effect; but this is rendered doubtful by the fact that experiments with sodium lactate consistently gave negative results.

Formaldehyde. The mutagenic potency of this substance had been discovered by Rapoport (1946) and confirmed by W. D. Kaplan (1948) and Auerbach (1949), working with *Drosophila*. Clear-cut positive results were obtained in our experiments also. Auerbach's hypothesis that production of mutations by formaldehyde can occur only through some intermediate effect on substances contained in *Drosophila* food is not supported by our experiments, in which the treatment was carried out on washed bacteria.

Phenol. The mutagenic action of phenol had been discovered by Hadorn and Niggli (1946), working on *Drosophila* with a very special treatment technique. Other techniques had failed to give positive results. Negative results had been obtained by Dickey, Cleland, and Lotz (1949) with *Neurospora*. Our tests gave clear-cut positive results for this substance, and also for two closely related substances, alpha-dinitrophenol and trinitro-

TABLE 1
RESULTS OF EXPERIMENTS TESTING INDUCTION OF BACK-MUTATIONS IN S4-4 (STREPTOMYCIN-DEPENDENT) E. COLI
TREATED WITH CERTAIN CHEMICALS

Chemical	Concentration %	Treatment (hours)	Survival %	Treated			Control		
				No. of plates	Total no. of bacteria	Mutants per 10 ⁸ bacteria	No. of plates	Total no. of bacteria	Mutants per 10 ⁸ bacteria
Sulfuric acid	0.003	3	13.	10	1.2 × 10 ⁸	11.8	4	8.5 × 10 ⁷	8.3
	0.002	3	12.	8	8.0 × 10 ⁷	70.0	4	7.0 × 10 ⁷	147.
	0.002	3	11.	8	1.1 × 10 ⁸	28.7	4	8.4 × 10 ⁷	35.4
	0.005	3	7.0	9	4.6 × 10 ⁷	10.9	4	5.4 × 10 ⁷	33.1
	0.002	3	1.9	8	2.2 × 10 ⁷	74.	4	9.2 × 10 ⁷	98.1
Phosphoric acid (ortho-)	0.008	3	29.	8	1.9 × 10 ⁸	29.4	4	6.4 × 10 ⁷	35.9
	0.008	3	17.	8	1.0 × 10 ⁸	16.5	4	7.3 × 10 ⁷	12.3
	0.010	3	1.2	8	9.3 × 10 ⁷	2.2	4	8.9 × 10 ⁷	21.3
Nitric acid	0.008	3	8.8	10	8.1 × 10 ⁷	11.1	4	8.5 × 10 ⁷	8.3
	0.005	3	8.3	8	5.8 × 10 ⁷	90.2	4	7.0 × 10 ⁷	147.
	0.005	3	6.9	8	6.8 × 10 ⁷	16.2	4	8.4 × 10 ⁷	35.4
	0.006	3	3.5	10	2.6 × 10 ⁷	11.7	4	5.4 × 10 ⁷	33.1
	0.005	3	2.7	8	2.2 × 10 ⁷	111.1	4	9.2 × 10 ⁷	98.1
Hydrochloric acid	0.0015	3	100.	5	6.0 × 10 ⁷	6.7	4	4.8 × 10 ⁷	10.4
	0.0015	3	33.	5	4.0 × 10 ⁸	5.8	4	9.6 × 10 ⁷	24.0
	0.00375	3	21.	5	2.5 × 10 ⁸	6.8			24.0
	0.0075	3	11.	10	1.3 × 10 ⁷	30.1			10.4
	0.0075	3	0.8	3	1.1 × 10 ⁷	0.0	2	9.2 × 10 ⁷	3.6
	0.0015	3	0.2	4	4.0 × 10 ⁸	0.0			3.6
	0.0015	3	0.2	4	3.8 × 10 ⁸	25.0	5	2.5 × 10 ⁸	26.2
Boric acid	4.0	3	50.	8	1.0 × 10 ⁸	27.3	6	7.6 × 10 ⁷	6.6
	1.8	24	33.	8	6.0 × 10 ⁸	28.9	5	1.1 × 10 ⁸	7.9
	2.0	24	25.	8	4.6 × 10 ⁸	28.7			7.9
	2.0	24	24.	8	3.3 × 10 ⁸	82.3	6	1.0 × 10 ⁸	5.8

TABLE 1—continued

Chemical	Concentration %	Treatment (hours)	Survival %	Treated			Control		
				No. of plates	Total no. of bacteria	Mutants per 10 ⁸ bacteria	No. of plates	Total no. of bacteria	Mutants per 10 ⁸ bacteria
Boric acid (continued)	2.0	24	17.	8	1.1 × 10 ⁸	6.6	6	4.6 × 10 ⁸	9.8
	2.0	24	11.	8	1.6 × 10 ⁸	64.1	6	1.0 × 10 ⁸	13.6
	1.5	24	11.	8	8.3 × 10 ⁷	31.2	4	8.9 × 10 ⁷	21.3
	1.7	24	10.	9	5.9 × 10 ⁷	20.3	6	9.0 × 10 ⁷	6.7
	2.0	48	9.7	8	9.2 × 10 ⁷	16.4	4	7.0 × 10 ⁷	5.7
	1.9	24	3.3	10	2.2 × 10 ⁷	18.6			6.7
	1.8	24	2.8	8	2.1 × 10 ⁷	56.6	4	7.5 × 10 ⁷	30.6
	0.013	3	86.	10	2.6 × 10 ⁸	11.7	5	3.0 × 10 ⁸	18.9
Sodium hydroxide	0.015	3	37.	10	3.0 × 10 ⁸	32.4	4	6.4 × 10 ⁷	35.9
	0.02	3	27.	7	5.4 × 10 ⁸	13.8	5	1.4 × 10 ⁸	16.8
	0.011	3	24.	10	3.4 × 10 ⁸	3.3	6	1.9 × 10 ⁸	3.6
	0.0145	3	20.	10	1.9 × 10 ⁸	20.2	4	8.5 × 10 ⁷	8.3
	0.015	3	16.	10	1.2 × 10 ⁸	16.3	4	7.3 × 10 ⁷	12.3
	0.0125	3	12.	8	1.2 × 10 ⁸	28.2	4	1.0 × 10 ⁸	38.0
	0.0125	3	11.	8	1.0 × 10 ⁸	130.7	4	9.2 × 10 ⁷	98.1
	0.014	3	5.7	8	5.3 × 10 ⁷	43.0	4	6.8 × 10 ⁷	43.8
	0.022	3	1.3	8	2.3 × 10 ⁷	21.6	5	1.1 × 10 ⁸	12.1
	0.00945	3	44.	10	2.8 × 10 ⁸	10.3	6	9.0 × 10 ⁷	6.7
	0.015	3	42.	8	3.8 × 10 ⁸	55.5	4	9.2 × 10 ⁷	98.1
	0.016	3	32.	8	3.0 × 10 ⁸	43.0	4	6.8 × 10 ⁷	43.8
Potassium hydroxide	0.015	3	29.	8	2.0 × 10 ⁸	79.2	4	7.0 × 10 ⁷	147.
	0.019	3	20.	10	3.6 × 10 ⁸	12.5	6	1.8 × 10 ⁸	5.5
	0.018	3	18.	10	1.4 × 10 ⁸	9.9	6	1.1 × 10 ⁸	9.7
	0.018	3	19.	10	3.4 × 10 ⁸	20.1			5.5
	0.0185	3	13.	10	1.9 × 10 ⁸	6.4	6	1.9 × 10 ⁸	3.6
	0.0165	3	13.	10	1.8 × 10 ⁸	4.4			3.6
	0.017	3	10.	10	7.9 × 10 ⁷	12.7			9.7

TABLE 1 (continued)

Chemical	Concentration %	Treatment (hours)	Survival %	Treated			Control		
				No. of plates	Total no. of bacteria	Mutants per 10 ⁸ bacteria	No. of plates	Total no. of bacteria	Mutants per 10 ⁸ bacteria
Silver nitrate (continued)	0.00025	3	59.	9	2.3 × 10 ⁸	2.1			2.3
	0.000100	3	26.	9	3.8 × 10 ⁸	7.4			8.6
	0.000100	24	4.3	9	6.1 × 10 ⁷	8.2			8.6
Copper sulfate	0.00020	3	88.	9	1.4 × 10 ⁸	5.7	6	1.0 × 10 ⁸	5.1
	0.00040	3	34.	9	4.7 × 10 ⁸	3.6	8	1.2 × 10 ⁸	8.8
	0.00050	3	22.	10	5.0 × 10 ⁸	4.4	5	1.1 × 10 ⁸	8.9
	0.00050	3	14.	9	3.8 × 10 ⁸	10.6	5	1.5 × 10 ⁸	2.1
	0.00050	3	12.	3	1.1 × 10 ⁸	20.7	4	1.2 × 10 ⁸	20.8
	0.00075	3	2.4	9	3.7 × 10 ⁷	40.5			5.1
	0.00100	3	0.36	9	7.2 × 10 ⁸	55.5			8.9
	0.00125	3	0.22	10	4.9 × 10 ⁸	0.0			8.9
Sodium silicate	0.025	3	66.	10	1.7 × 10 ⁸	5.9	5	1.3 × 10 ⁸	6.3
	0.100	3	33.	5	4.2 × 10 ⁸	2.4	5	1.3 × 10 ⁸	5.3
	0.050	3	27.	10	6.9 × 10 ⁷	8.7			6.3
	0.100	3	16.	7	3.8 × 10 ⁸	6.6	5	1.7 × 10 ⁸	6.1
	0.100	3	4.6	9	7.9 × 10 ⁷	11.4	7	1.3 × 10 ⁸	6.2
	0.150	3	3.0	9	5.1 × 10 ⁷	2.0			6.2
	0.300	3	0.11	5	1.3 × 10 ⁸	0.0	5	1.2 × 10 ⁸	7.0
Acetic acid	0.03	3	79.	10	1.3 × 10 ⁸	8.3	5	1.6 × 10 ⁸	10.0
	0.03	3	36.	8	9.9 × 10 ⁸	36.4	5	1.7 × 10 ⁸	9.2
	0.03	3	11.	10	8.5 × 10 ⁷	15.3			10.0
	0.01	24	7.2	8	7.6 × 10 ⁷	19.8	6	7.9 × 10 ⁷	10.1
	0.03	3	6.0	10	9.5 × 10 ⁷	25.2			10.0
	0.03	3½	0.9	8	8.8 × 10 ⁸	56.8	6	7.6 × 10 ⁷	6.6
Lactic acid	0.010	3	40.	10	6.4 × 10 ⁸	12.8	5	1.6 × 10 ⁸	10.0
	0.010	3	36.	10	2.9 × 10 ⁸	10.7			10.0
	0.019	3	14.	8	2.0 × 10 ⁸	16.7	5	8.9 × 10 ⁷	4.5

TABLE 1 (continued)

Chemical	Concentration %	Treatment (hours)	Survival %	Treated			Control		
				No. of plates	Total no. of bacteria	Mutants per 10 ⁸ bacteria	No. of plates	Total no. of bacteria	Mutants per 10 ⁸ bacteria
Lactic acid (continued)	0.021	3	7.5	8	1.1×10^8	40.2	5	1.6×10^8	4.5
	0.020	3	6.8	8	1.7×10^8	17.0	5	9.2×10^7	15.3
	0.020	3	4.6	8	6.7×10^8	32.9	5	8.5×10^7	27.3
	0.020	4	2.5	8	2.9×10^7	34.7	6	1.0×10^8	4.8
	0.020	3	1.9	8	2.6×10^7	11.4	5	1.1×10^8	12.1
Sodium lactate	2.5	3	85.	8	1.5×10^8	7.3	5	1.6×10^8	15.3
	3.0	3	61.	8	1.7×10^8	16.4	5	7.9×10^7	10.1
	2.0	24	8.3	8	8.7×10^7	5.8	5	1.7×10^8	9.2
	2.5	24	1.4	8	3.8×10^7	5.2	6	1.1×10^8	2.7
	0.0070	3	100.	8	1.5×10^8	18.1	5	1.1×10^8	12.1
Formic acid	0.0060	3	93.	8	1.4×10^8	18.4	5	1.7×10^8	9.2
	0.0050	3	77.	8	1.3×10^8	20.2	5	1.6×10^8	15.3
	0.0065	3	30.	8	8.2×10^8	44.0	5	1.5×10^8	3.3
	0.0060	3	20.	8	5.0×10^8	43.3	6	7.1×10^7	2.8
	0.0065	3	19.	8	4.6×10^8	24.4	5	1.3×10^8	26.2
	0.0060	3	15.	8	1.4×10^8	27.7	4	9.4×10^8	15.5
	0.0075	3	3.4	8	8.2×10^7	38.0	4	7.6×10^7	79.
	0.0070	3	2.8	8	2.6×10^7	38.5	4	7.6×10^7	68.
	0.01	3	61.	2	7.6×10^7	400.	4	7.3×10^7	12.3
	0.01	3	58.	5	6.8×10^8	133.	4	1.5×10^8	15.5
Formaldehyde	0.02	3	58.	5	6.8×10^8	157.	4	7.6×10^7	79.
	0.01	3	48.	4	3.7×10^7	245.	4	7.6×10^7	68.
	0.01	3	48.	4	3.7×10^8	470.	4	7.3×10^7	12.3
	0.01	3	21.	8	1.3×10^8	470.	4	7.3×10^7	12.3
	0.02	3	16.	8	1.0×10^8	129.	4	7.3×10^7	12.3
	0.04	3	9.0	7	1.5×10^8	326.	7	1.5×10^8	15.5
	0.05	3	1.7	10	3.2×10^8	560.	10	1.5×10^8	79.

TABLE 1 (continued)

Chemical	Concentration %	Treatment (hours)	Survival %	Treated			Control		
				No. of plates	Total no. of bacteria	Mutants per 10 ⁸ bacteria	No. of plates	Total no. of bacteria	Mutants per 10 ⁸ bacteria
Phenol	0.10	3	90.	10	4.4×10^8	84.5	4	2.0×10^8	74.5
	0.10	3	69.	9	1.2×10^8	2.5	5	9.5×10^8	0.6
	0.08	3	48.	8	7.4×10^8	3.4			0.6
	0.20	3	43.	9	7.5×10^8	2.5			0.6
	0.10	12	1.7	10	7.3×10^8	124.	5	2.2×10^8	74.8
	0.10	24	1.1	10	3.1×10^8	192.	4	1.1×10^8	59.5
	0.20	3	0.5	9	2.3×10^8	820.			74.5
Alpha- dinitro- phenol	0.005	3	87.	3	9.6×10^8	2.7	2	7.4×10^7	4.1
	0.020	3	72.	3	1.1×10^8	8.1	5	2.5×10^8	5.5
	0.050	3	58.	10	1.7×10^8	33.5	5	1.5×10^8	5.4
	0.020	3	46.	10	9.5×10^7	51.5	4	8.4×10^7	8.5
	0.005	3	37.	10	2.3×10^8	25.1	5	3.1×10^8	12.5
	0.050	3	32.	10	6.6×10^7	56.0			8.5
	0.025	3	31.	9	1.7×10^8	14.2			12.5
	0.040	3	23.	10	4.8×10^7	25.0			8.5
	0.050	3	18.	10	9.1×10^7	37.4			5.5
	0.100	3	3.0	9	8.2×10^7	44.0	5	1.5×10^8	7.2
	0.100	3	2.6	10	7.3×10^7	97.0	5	1.4×10^8	7.3
	0.100	3	2.5	9	1.2×10^8	6.0			5.5
	0.100	3	2.4	7	3.5×10^7	65.5	4	8.4×10^8	7.6
	0.100	3	1.7	8	4.8×10^7	79.0	4	1.4×10^8	2.8
	0.010	3	94.	8	1.9×10^8	14.9	5	1.3×10^8	14.3
Trinitro- phenol	0.018	3	34.	8	4.9×10^8	97.3	6	1.1×10^8	9.3
	0.017	3	7.4	8	5.8×10^7	132.	6	5.8×10^7	3.4
	0.017	3	4.2	8	6.2×10^7	27.4	5	8.9×10^7	4.5
	0.018	3	1.9	8	1.9×10^7	68.4	6	7.8×10^7	7.7

TABLE 1 (continued)

TABLE 1 (continued)

Chemical	Concentration %	Treatment (hours)	Survival %	Treated			Control		
				No. of plates	Total no. of bacteria	Mutants per 10 ⁸ bacteria	No. of plates	Total no. of bacteria	Mutants per 10 ⁸ bacteria
Carbamate (n-propyl) (continued)	2.10	3½	3.9	8	8.1 × 10 ⁷	9.9			3.9
	2.10	3	2.9	8	7.0 × 10 ⁷	24.2			3.3
	2.10	3½	2.5	8	3.2 × 10 ⁷	34.8	6	9.7 × 10 ⁸	12.3
	2.10	4	1.4	8	3.4 × 10 ⁷	23.8			3.3
Carbamate (n-butyl)	0.50	3	85.	8	8.6 × 10 ⁸	21.4	6	7.8 × 10 ⁷	7.7
	0.76	3½	44.	7	9.2 × 10 ⁸	5.3	6	1.8 × 10 ⁸	2.2
	0.75	3½	29.	8	7.0 × 10 ⁸	5.6	5	1.5 × 10 ⁸	3.3
	0.75	3½	20.	8	4.2 × 10 ⁸	8.3	6	1.6 × 10 ⁸	3.9
	0.75	3½	19.	7	1.7 × 10 ⁸	20.9	6	7.6 × 10 ⁷	6.6
	0.75	3½	17.	8	2.6 × 10 ⁸	7.4	6	1.1 × 10 ⁸	2.7
	0.75	3	16.	8	1.6 × 10 ⁸	10.0	7	7.6 × 10 ⁷	9.2
	0.80	3½	4.9	8	1.2 × 10 ⁸	5.9			3.3
	0.75	3½	3.4	8	4.5 × 10 ⁷	8.9	6	9.7 × 10 ⁷	12.3
	0.75	3½	1.4	8	1.5 × 10 ⁷	0.0	6	7.8 × 10 ⁷	6.4
	0.25	3	100.	8	2.0 × 10 ⁸	8.4	6	1.6 × 10 ⁸	3.9
	0.30	3	100.	8	1.6 × 10 ⁸	11.3	6	1.1 × 10 ⁸	2.7
Carbamate (isoamyl)	0.35	3	57.	8	8.5 × 10 ⁸	8.0			2.7
	0.35	3	54.	8	1.1 × 10 ⁸	9.4			3.9
	0.36	3	26.	8	6.2 × 10 ⁸	6.3	6	1.8 × 10 ⁸	2.2
	0.38	3	6.3	8	1.5 × 10 ⁸	7.2			2.2
	0.37	3	5.8	8	1.4 × 10 ⁸	7.0			2.2
	0.00025	3	50.	9	7.6 × 10 ⁸	57.3	9	1.5 × 10 ⁸	6.6
Neutral red (in light)	0.00100	3	43.	9	6.6 × 10 ⁸	118.			6.6
	0.00050	3	24.	8	3.2 × 10 ⁸	122.			6.6
	0.00050	12	0.2	8	1.2 × 10 ⁸	178.	8	6.7 × 10 ⁸	10.8
	0.00050	24	90.	9	4.2 × 10 ⁸	17.2	6	4.6 × 10 ⁸	10.1
Neutral red (in the dark)	0.00050	12	86.	8	9.6 × 10 ⁷	10.6	7	9.8 × 10 ⁷	9.8

TABLE 1 (continued)

Chemical	Concentration %	Treatment (hours)	Survival %	Treated		Control			
				No. of plates	Total no. of bacteria	Mutants per 10 ⁸ bacteria	No. of plates	Total no. of bacteria	Mutants per 10 ⁸ bacteria
Neutral red (in the dark) (continued)	0.00050	24	83.	12	6.5 × 10 ⁷	15.4	10	6.5 × 10 ⁷	15.4
	0.00100	24	71.	10	4.6 × 10 ⁷	17.4			15.4
	0.00050	12	70.	15	9.0 × 10 ⁸	10.8	8		10.8
Acridiflavine	0.0015	3	39.	10	4.8 × 10 ⁸	78.3	4	1.0 × 10 ⁸	14.4
	0.0030	3	28.	10	3.4 × 10 ⁸	59.1			14.4
Caffeine alkaloid	2.10	3	72.	7	1.3 × 10 ⁸	15.5	6	7.6 × 10 ⁷	6.6
	0.60	48	44.	7	7.8 × 10 ⁸	31.1	6	1.5 × 10 ⁸	9.8
	2.10	24	34.	8	1.7 × 10 ⁸	30.3	6	3.7 × 10 ⁷	10.9
	0.90	48	15.	8	3.9 × 10 ⁸	21.8	5	1.1 × 10 ⁸	7.9
	1.00	48	15.	8	1.3 × 10 ⁸	32.1	6	6.7 × 10 ⁷	17.9
	0.50	48	12.	10	7.0 × 10 ⁷	13.0	6	7.1 × 10 ⁷	5.6
	0.60	48	5.1	8	9.3 × 10 ⁷	34.6			7.9
	1.09	48	3.8	8	2.0 × 10 ⁷	20.4	6	3.9 × 10 ⁷	2.5
	0.20	3	100.	8	4.6 × 10 ⁸	23.6	6	1.4 × 10 ⁷	6.9
	0.05	3	100.	8	4.2 × 10 ⁸	26.0			6.9
Necrosin	0.40	3	100.	8	1.2 × 10 ⁸	16.4	6	1.0 × 10 ⁸	9.9
	1.30	3	44.	8	8.5 × 10 ⁸	17.8	6	1.4 × 10 ⁸	8.4
	0.65	3½	3.4	7	3.9 × 10 ⁷	172.	6	9.8 × 10 ⁷	4.1

phenol (picric acid). The mutagenicity of phenol, however, was evident only after treatments that left very few survivors.

Sodium salicylate. Not mutagenic.

Carbamates. It was known from Oehlkers' work (1943) that some carbamates were able to induce chromosomal aberrations. Vogt (1948) had discovered that ethyl carbamate (urethane) showed mutagenic action in *Drosophila*. Since then, carbamates had been used by Bryson (unpublished) to induce mutations to phage resistance in *E. coli*. In our experiments, methyl carbamate gave negative results; ethyl and *n*-propyl carbamate gave good positive results; and *n*-butyl carbamate and isoamyl carbamate showed much weaker mutagenic effect.

Neutral red. Luther (1939) had found that neutral red was able to produce chromosomal aberrations. Buzzati (1947), treating *Drosophila* eggs with this substance, had observed an increase in frequency of mosaic flies. In our tests it seemed at first impossible to obtain low survival ranges, because treatment with concentrations higher than 0.001 per cent produced heavy clumping, and increases in length of treatment did not appreciably affect the number of survivors. Therefore no clear-cut positive results were obtained with the standard procedure. Since neutral red is a photodynamic substance, however, we then tried exposing the bacteria to light during treatment (a 60-watt bulb at a distance of about 10 cm), and finally got the desired survival ranges and with them a very large increase in frequency of mutants. Light had no effect on the controls. Similar results were reported by Kaplan (1948, 1949, 1950a, 1950b), treating *Bacterium prodigiosum* and *E. coli* with erythrosine.

Acridine. This substance was known to be a mutagenic agent for both bacteria and *Drosophila* (Witkin, 1947). Our tests also showed clear-cut positive results.

Caffeine. Caffeine had been found to be mutagenic for *Ophiostoma* (biochemical mutations) by Fries and Kihlman (1948) and for *E. coli* (mutations to phage resistance) by Witkin (Demerec, Wallace, and Witkin, 1948). Our experiments confirmed these results.

Necrosin. Necrosin is the name given by Menkin (1943) to the potent injurious euglobulin fraction of the exudate of inflamed tissues of vertebrates. A similar substance has subsequently been found by him in severely damaged tissues of some invertebrates (Menkin, 1949). The sample of necrosin used in our experiments was received from Dr. Menkin. It was found to be mutagenic, particularly in experiments where the proportion of survivors was low.

DISCUSSION

Streptomycin-dependent (Sd-4) bacteria, when plated on broth-agar medium without any streptomycin, undergo several divisions (residual growth) and then stop growing. The concentration of streptomycin in the medium in which the parent bacteria are grown is the most significant of the known factors influencing the number of divisions. For example, bacteria grown

in broth medium containing 10 micrograms of streptomycin per milliliter, when plated on medium without streptomycin, will pass through 2-3 divisions; whereas bacteria grown in medium containing 25 micrograms of streptomycin will pass through 3-4 divisions. It has been found that very few, if any, nondependent back-mutants (background mutants) are present in cultures grown in broth with streptomycin, and therefore almost all such back-mutants appearing on the streptomycin-free plates can be assumed to originate during the residual growth. The almost complete absence of background mutants reduces the possibility that differential killing may be responsible for the increased proportion of back-mutants found among the survivors of treatment with certain chemicals; and this, together with the facts that residual growth can be dependably regulated in untreated material and that it allows expression of the back-mutations induced by treatment, makes the Sd-4 method especially suitable for studies of induced mutability.

Since spontaneous back-mutations occur during residual growth, any factor that increases this growth should increase the number of mutants. Therefore, in experiments such as these described here, it is necessary to be sure that any observed increase in mutant frequency is an effect of the treatment on the treated bacteria and not an effect of the treatment on the extent of residual growth. It is not likely that the chemicals used in these experiments would increase the residual growth. In the first place, they were usually applied in nonphysiological, toxic doses. Furthermore, the streptomycin requirement of the dependent bacteria is highly specific (Demerec, 1950), and ability to substitute for it would be quite unexpected in an unrelated chemical. However, even supposing that this had occurred, it would have been detected, either by the appearance of heavy residual growth on the plates of treated bacteria or through the microscopic check of the extent of residual growth that was made in all doubtful cases.

The analysis of data on the mutation-producing chemicals, presented in table 2, indicates that there were considerable differences of behavior among them. Some (ammonia, copper sulfate, and necrosin) were mutagenic only in treatments that left less than 5 per cent survivors; whereas others (hydrogen peroxide, formaldehyde, alpha-dinitrophenol, and neutral red in light) showed effects in treatments that allowed more than 50 per cent survival. Formaldehyde appears to be the most potent mutagen on the list. A treatment that left 61 per cent survivors resulted in 400 mutants per 10^8 treated bacteria; and two other treatments, each leaving 58 per cent survivors, gave 133 and 157 mutants, whereas in the controls there were only 25.2 and 15.5 mutants per 10^8 .

Of the 31 chemicals listed in table 1, 19 were found to be mutagenic (table 2), and the following 12 were not: sulfuric, phosphoric, nitric, and hydrochloric acids; sodium and potassium hydroxides; mercuric chloride; silver nitrate; sodium silicate; sodium lactate; sodium salicylate; and methyl carbamate. Since the potency of the different mutagenic chemicals ranges from slight to strong, and, at least in the case of one mutagen that has been studied intensively (manganous chloride), can be shifted from one

TABLE 2
SUMMARY OF THE DATA ON MUTAGENIC CHEMICALS, SHOWING HIGHEST AND
LOWEST FREQUENCIES OF MUTANTS OBSERVED IN EXPERIMENTS USING
DIFFERENT TREATMENTS (EXPRESSED IN PERCENTAGES
OF SURVIVORS)

	Survivors (per cent)			Controls
	50-100	5-50	less than 5	
No. of mutants per 10 ⁸ bacteria				
Boric acid	27	16-82	19-57	6-31
Ammonia	5	2-10	38-121	3-13
Hydrogen peroxide	20-29	121-124	27-175	5-7
Copper sulfate	6	4-21	40-56	2-21
Acetic acid	8	15-36	57	7-10
Lactic acid	...	11-40	11-35	5-27
Formic acid	18-20	24-44	38-39	3-15
Formaldehyde	133-400	120-326	560	12-79
Phenol	3-85	...	124-820	1-75
Alpha-dinitrophenol	3-34	14-56	6-97	3-13
Trinitrophenol	15	9-132	27-68	3-14
Carbamate, ethyl	19-27	7-53	40-99	6-20
<i>n</i> -propyl	12-28	8-30	10-35	2-12
<i>n</i> -butyl	21	5-21	6-9	2-12
isoamyl	8-11	6	7	2-4
Neutral red, light	57	118-122	178	7-11
dark	11-17	7-15
Acridine	...	59-78	...	14
Caffeine	16	13-35	20	3-18
Necrosin	16-26	18	172	4-10

extreme to the other by simple pretreatments of the bacteria, it seems very likely that the chemicals listed above as nonmutagens might become mutagenic if the treatment were made in a certain way. At any rate, examination of the list of mutagens in the table shows that mutagenicity does not belong to any one group of chemicals but is distributed widely among the different groups tested. Chemicals with such different properties as boric acid, ammonia, hydrogen peroxide, copper sulfate, acetic acid, formaldehyde, and phenol are on the list of mutagens. This fact, together with the indicated possibility that chemicals now considered noneffective would become mutagenic if appropriate conditions of treatment were found, leads us to conclude that genetic changes may be induced by most agents that are able to enter a living cell, upsetting some of its metabolic functions. It seems very likely that the genetic effect of a chemical is not direct but induced through changes in the cytoplasm, which in turn affect the genes. If this is so, it should be very difficult to establish what is the final reaction producing a change in a gene. It may well be that similar final reactions may be initiated by very different primary changes.

In this study we were considering back-mutations from Sd-4; and there is evidence to indicate that a majority of these may occur at one gene locus (Demerec, 1950; Newcombe and Nyholm, 1950). Several experiments by

others, however, based on observation of mutations to T1 phage resistance, have tested the mutagenicity of a few of the chemicals we used. Witkin (Witkin, 1947; Demerec, Wallace, and Witkin, 1948) found that acriflavine and caffeine were mutagenic; and Bryson (in manuscript), in extensive experiments with several carbamate compounds, showed that ethyl carbamate (urethane) is mutagenic. It therefore appears very probable that the mutagenic effect observed by us was not specific, but that treatment with these chemicals increases the mutation rate of the whole genome.

It should be kept in mind that we are dealing here with genetic changes that occur spontaneously with a rate of the order of one per 10^8 cell generations, a considerably lower rate than could be detected directly in germ cells of multicellular organisms. Since each cell of a multicellular organism is a genetic entity, however, in which mutations may occur spontaneously or be induced by various agents, the likelihood of mutation in some of the somatic cells of a higher organism is good, if a large number of these cells are exposed to the action of a mutagenic chemical. This likelihood is further increased by the fact that each cell contains several thousand genes, in any one of which a mutation may be induced.

SUMMARY

Thirty-one chemicals, representing various organic and inorganic groups, were tested for ability to induce back-mutations from streptomycin dependence (Sd-4) to nondependence in *E. coli*. Nineteen were found to be mutagens. It was demonstrated that mutagenicity is not a specific property of any one group of chemicals, but appears among widely different groups. Chemicals having such different properties as boric acid, ammonia, hydrogen peroxide, copper sulfate, acetic acid, formaldehyde, and phenol were found to be mutagenic, indicating that genetic changes may be induced by many agents that are able to enter the living cell and upset its metabolic functions.

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AN INSTANCE OF AMIXIA BETWEEN TWO SPECIES OF
LANDSNAILS (PULMONATA, HELMINTHOGLYPTIDAE)

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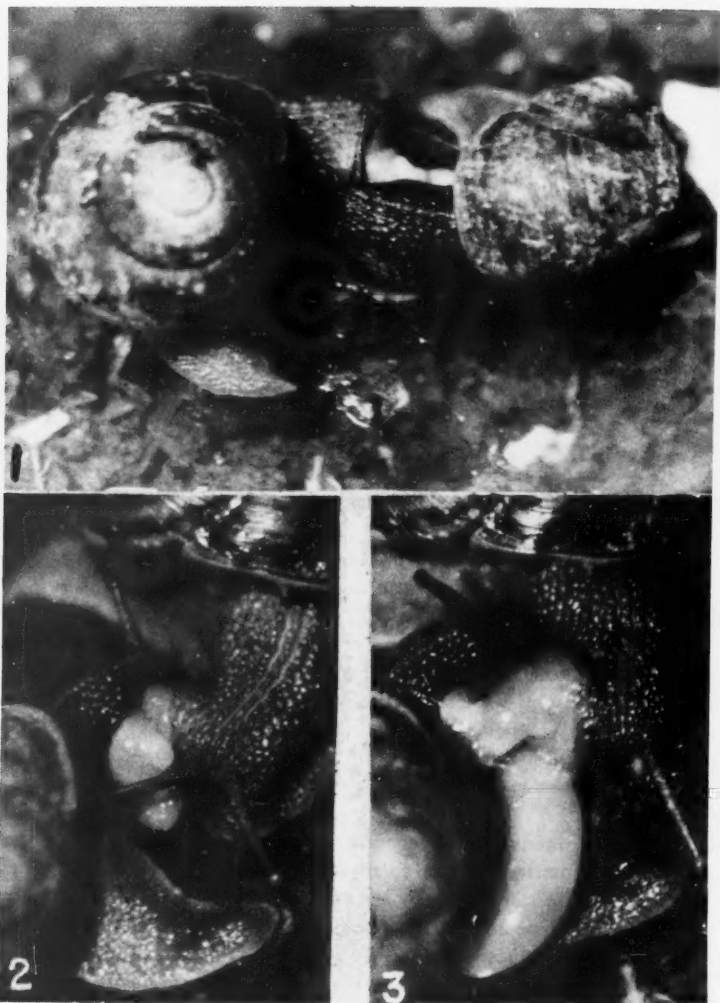
When one surveys the variants of the basic type of sex-organ exhibited by the numerous forms of Helminthoglypta, (Pilsbry, 1939) the functional properties of these organs as either panmictic or amictic forces of evolution present an interesting problem. The following data bear on the sexual isolation of two species, *Helminthoglypta californiensis vineta* (Valenciennes) and *H. dupetithouarsi* (Deshayes).

The specimens of *californiensis* were collected at the dumps north of Monterey, California, with E. P. Chace, who kindly showed me the special habitat preference of the species. The specimens of *dupetithouarsi* were secured from the pine-forested hills above Monterey. The lots of the two species were being kept together in a carton cage when the following observations were made.

A large *dupetithouarsi* was noted following at the tail-tip of a *californiensis* and, throughout the observations, was the more amorous of the pair. After the *dupetithouarsi* probed its dart into the side of the foot of the other species, the *californiensis* turned toward the aggressor and exerted the penis and atrium, as also did the *dupetithouarsi*. Copulation was not attempted, however, and the *californiensis* began to disengage from the courtship.

Since courting landsnails frequently wander apart but rejoin and finish mating, I have found it useful to reposition errants so that observations can be completed or mating-anatomies secured. Accordingly, the seemingly separating snails were artificially repositioned to determine if mating would continue and copulation eventuate. By gently picking the two snails up and placing them on a small, wet, glass plate, it was possible to gently slide the snails into the head-on position again whenever they had started separating. By such maneuvers the pair were kept in courtship, until it culminated in attempts at coitus. However the penis tip (verge) of the *dupetithouarsi*, although frequently protruded vigorously, failed to penetrate the orifice of the female organ of the *californiensis* and the latter's penis proved to be too short to reach the female orifice of the former.

An entire view of the attempted mating is shown in figure 1, the onset in figure 2, and the details of the attempted coitus in figure 3, in which the curved, finger-like dart-organ of the *dupetithouarsi* is in functioning position against the foot of the other snail, and the tapering upper lobe is the penis



FIGURES 1-3. The attempted coition between *Helminthoglypta dupetitthouarsi* (Deshayes) and *H. californiensis vincta* (Valenciennes). The anatomical details are shown in figures 2-3 at greater enlargement (see text).

attempting ingress into the female organ of the *californiensis*. In figure 1 the snail to the left is the specimen of *Helminthoglypta dupetitthouarsi* and the smaller snail to the right is the specimen of *Helminthoglypta californiensis*.

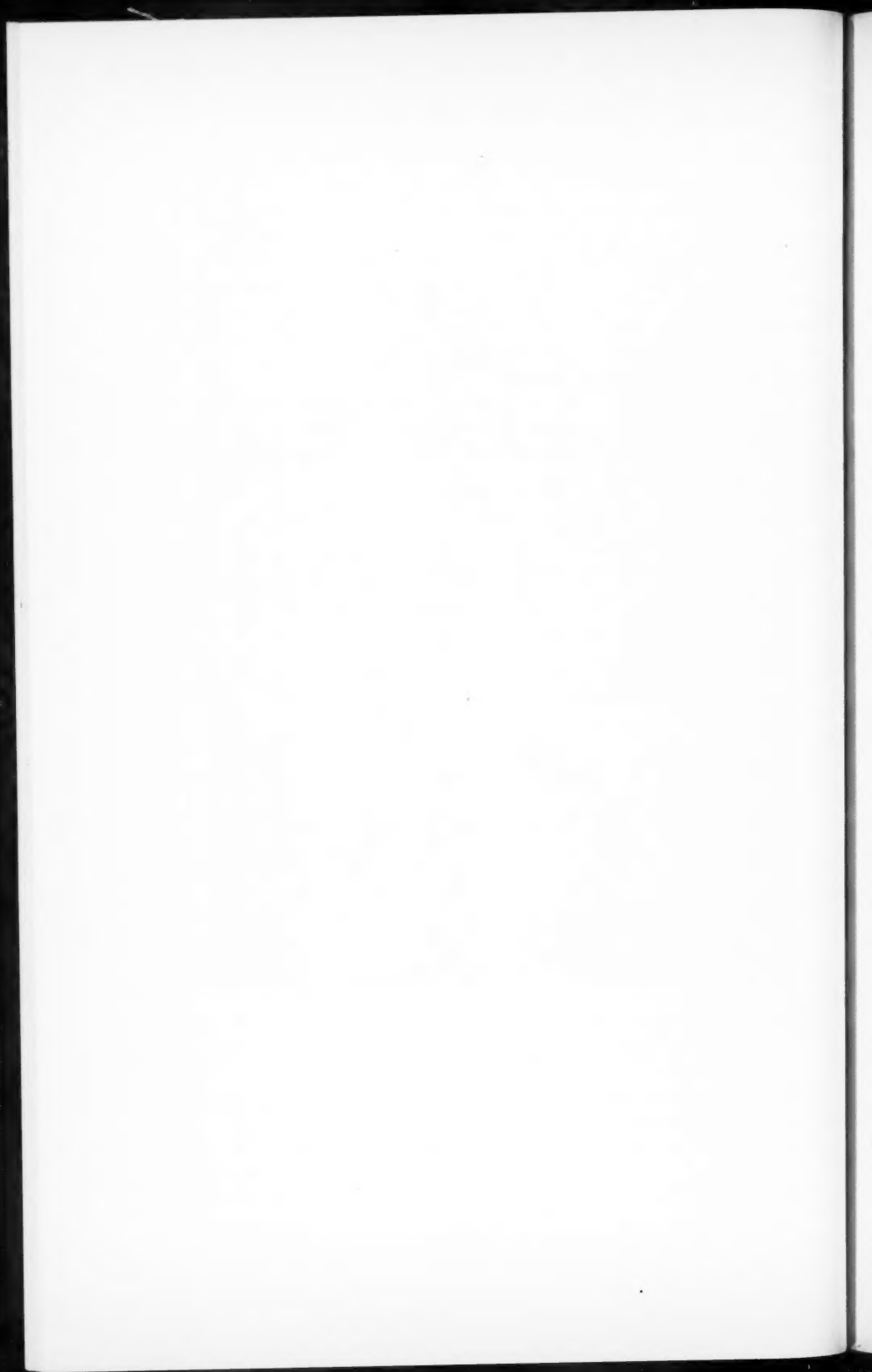
The observations ended when the *californiensis* retracted its sex-organs and turned away. It was seen to bear several deep (2-3 mm.) dart-wounds on the sole of the foot. Although generally present in recently copulated

specimens of *Helminthoglypta*, the wounds in this individual seemed more conspicuous and were still evident the next day. On the fourth day after the attempted mating, the *californiensis* was found dead with its foot noticeably swollen. The primary cause of death seems to have been the dart-wounds, which may secondarily have become much infected. Having never previously noted dart-wounds to be mortiferous in *Helminthoglypta* subsequent to matings, it is possible that the mucus from the dart of the *dupetitboursi* was harmful to the tissues of the other species, or that the nearly three-times-longer dart provided a path for infection into tissues not ordinarily penetrated or wounded.

I am much indebted to Dr. S. Charles Kendeigh of the University of Illinois for helpful suggestions in writing this paper. Further observations on the sexology of these species have been published elsewhere (Webb, 1942).

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

A SUPPRESSOR MUTATION IN *ESCHERICHIA COLI**

Nutritionally deficient mutant strains of bacteria (auxotrophs) are often capable of undergoing "reversion," giving rise to strains having the wild type nutritional state (prototrophs). It is usually impossible to determine whether the prototrophs are true back-mutants, or whether the wild phenotype is restored by the action of an independent suppressor mutation.

An auxotroph requiring histidine was isolated from strain B/r of *Escherichia coli* (which grows well on minimal medium) after ultraviolet irradiation and layering.¹ Some time later, the histidineless strain was irradiated with ultraviolet and subjected to penicillin screening.^{2,3} Among the mutants isolated was one requiring serine or glycine in addition to histidine. This diauxotroph, strain M2, was thus characterized by two growth factor requirements acquired separately and serially, presumably by two mutational steps.

A study of the reversion behavior of strain M2 revealed an interesting departure from the usual result with diauxotrophs. Prototrophs were obtained with a frequency of about $1/10^7$ bacteria plated in minimal medium. When the strain was plated in minimal medium supplemented with histidine, or with serine, prototrophs were obtained in addition to the expected single reversions, and the rate of double reversion was found to be about equal to the rate of single reversion from either of the separate requirements. The prototrophs obtained, therefore, could not possibly result from coincidental back-mutation of the two independent requirements. The quantitative results strongly suggested a suppressor, as did the fact that the growth rate of the prototrophs obtained from M2 was considerably slower than that of the wild type parent strain in minimal medium.

The suppressor hypothesis was tested by attempting to induce reversion of the postulated suppressor, thereby releasing the double requirement for histidine and serine or glycine in a single step. A prototroph strain, M2P, obtained from a plating of M2 on minimal medium, was irradiated with ultraviolet light and put through the penicillin procedure. Mutants were screened on minimal plates supplemented with both serine and histidine, so that any variant requiring one or both of these growth factors could be detected. Colonies appearing on these plates were analyzed, and eight out of 130 tested proved to require *both* histidine and serine or glycine. As a control, the wild type strain was irradiated and penicillin-screened in exactly the same way. Three hundred twenty colonies appearing on the serine-histidine supplemented plates were analyzed, and none was found to require both growth factors. These results can best be interpreted by

assuming that the prototrophs arising in strain M2 owe their wild phenotype to the action of an ultraviolet-reversible suppressor, capable of overcoming the two independent metabolic blocks. The biochemical basis of this system is obscure, but a possible explanation could be the following: (1) the mutation to histidine requirement blocks histidine synthesis by the production of an inhibitor; (2) the mutation to serine or glycine requirement blocks serine-glycine synthesis by an increase in the concentration of the same inhibitor; (3) the suppressor mutation eliminates or otherwise neutralizes the inhibitor.

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